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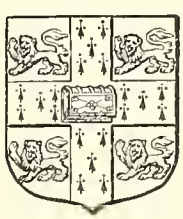

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ON TWO COLLECTIONS OF INDIAN TICKS.

BY CECIL WARBURTON, M.A.,
Zoologist to the Royal Agricultural Society.

(*From the Quick Laboratory, University of Cambridge.*)

(With 10 Text-Figures.)

A SMALL but extremely interesting collection of Ticks and other Arachnids was brought over from Ceylon by Mr C. C. Dobell in 1909, and was supplemented by a few tubes sent subsequently by Dr A. Willey from the same island. While these were being examined, a large consignment of ticks was received from the Indian Museum, Calcutta, being in fact the whole collection of the museum. The two collections may be conveniently dealt with in the present paper, which will give a list of the species they respectively contain, and a description of the new forms—five new species and three new varieties—which they jointly afford.

The Dobell-Willey collection owes its chief interest, as regards its Ixodidae, to the fact that it contains the only parasites hitherto reported as taken from the Mouse Deer, *Tragulus meminna*. These comprise two species of *Haemaphysalis*, both new, and one of them very remarkable in that it does not conform to one of the most constant characters of the genus, its palps being very much longer than broad.

The Indian Museum collection, drawn from widely separated localities and from many hosts, naturally includes a considerable number of species, two of which are new. It has also attracted special attention to the species *Aponomma gervaisi*, of which there seem to exist two distinct forms, one of which has been relegated to a new variety.

Ticks contained in the Dobell-Willey collection:

1. *Haemaphysalis longipalpis*, n. sp., from the Mouse Deer, *Tragulus meminna*, Ceylon.
2. *H. cuspidata*, n. sp., from the same host. Some nymphs taken from *Paradoxurus niger* at Colombo by Dr Willey were also attributed to this species.
3. *Aponomma gervaisi* (type variety), Lucas, 1847, from *Varanus bengalensis*, *Coclotes versicolor*, *Zamenis mucosus* and *Naia tripudians*.
4. *A. laeve*, Neumann, 1899, from *Zamenis mucosus*.

Ticks contained in the Indian Museum collection, Calcutta:

Argas persicus, Oken, 1818. Indian Museum Library.
A. vespertilionis, Latreille, 1796. Belgaum, Bombay.
A. reflexus var. *indicus*, n. var. Indian Museum Buildings.
Ixodes gigas, n. sp. Punkabani, host unknown.
Haemaphysalis bispinosa, Neumann, 1897. From dog, tiger, Macacus, in various localities.
H. flava, Neumann, 1897. From sheep, Simla Hills.
H. hystricis, Supino, 1897. From *Geocmyda spinosa*, ? Burma.
H. leachi var. *australis*, Neumann, 1905. *Felis tigris*.
H. leachi var. *indica*, n. var. From *Canis aureus*, Museum Compound.
[*Dermacentor rhinocerotis*, De Geer, 1778. Rhinoceros, Ketloa, Abyssinia.]
Rhipicephalus sanguineus, Latreille, 1804. From *Canis* spp. and *Erinaceus* spp. in many localities.
R. haemaphysaloides, Supino, 1897. From *Felix marmorata*, Zoological Gardens, Calcutta, and from other localities.
R. brevieeps, n. sp. From *Erinaceus collaris*, Sind.
Hyalomma aegyptium, Linnaeus, 1758. From *Cervus affinis* and several species of *Erinaceus* in various localities.
H. syriacum, Koch, 1844. From land tortoise, Quetta, Baluchistan.
Amblyomma testudinarium, Koch, 1844–7. From Sikkim and from Sibsagar, Assam.
A. decoratum, Koch, 1844–7. From *Geoemyda grandis*, Zoological Gardens, Calcutta, and from *Varanus salvator*, Nicobars.
A. sublaeve, Neumann, 1899. From *Manis* spp. and *Euprepes* in many localities.
A. annandalei, n. sp. From *Geoemyda spinosa*, "India (Burma)."
Aponomma gervaisi, Lucas, 1847. From *Varanus* spp., Agra, Karachi, and from *Naia tripudians*, Calcutta.
A. gervaisi var. *lueasi*, n. var. From various reptiles in the Zoological Gardens, Calcutta.
[*A. deeorosum*, L. Koch, 1867. From *Varanus salvator*, Brisbane, Australia.]

The species included in brackets are extra-Asiatic.

ARGAS REFLEXUS var. INDICUS, n. var.

Differs from the type in the slightly more terminal position of the capitulum, the smaller coxae, and especially the absence of the dorsal protuberance on the 4th tarsus.

The Indian Museum collection contained three specimems of this form. One was found in a book in the Entomological room, and another on a wall of the Museum building. The third specimen was found in a box sent from the Museum to Kurseong, E. Himalayas.

The birds building in the roof of the Museum were identified as *Passer domesticus* and *Cypselus affinis*. The type species has not been recorded from India.

IXODES GIGAS, n. sp.

(Figs. 1 and 2.)

Male. 5 × 3 mm. (capitulum excluded). Body oval, broadest near the hind end.

Scutum. Leaving a broad fold at the sides and at the posterior border; chestnut-coloured, darker on scapulae and sides, glossy and smooth except for a few small punctations on the scapulae and between the cervical grooves. Cervical grooves parallel at first, then sharply diverging; no lateral grooves.

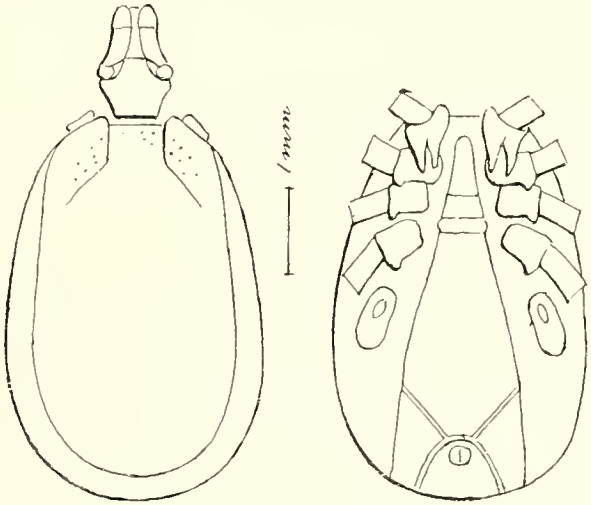

Fig. 1. Fig. 2.

Fig. 1. *Ixodes gigas*, ♂. Dorsal aspect.
Fig. 2. *Ixodes gigas*, ♂. Ventral aspect.

Capitulum moderate. Base rather long, pentagonal, without cornua. Palps of medium length, the first article rather prominent laterally, the second article about twice the length of the third. Hypostome 2 | 2, seven or eight teeth per file.

Venter. Pregenital shield elongate, rather indented anteriorly. Genital grooves gently diverging to reach the posterior border. Anus rather near the posterior border, the anal groove with the sides curved and slightly diverging. Genital orifice between coxae III and IV. Spiracles rather large, oval or slightly reniform. *Legs* long. Coxae I bidentate, like those of a *Rhipicephalus* or *Hyalomma*, protruding in front of the body and visible dorsally. Coxae II—IV with a slight external tooth and a more blade-like internal edge. All the coxae close together and occupying little more than the anterior third of the length of the body. Tarsi long and tapering, only slightly gibbous dorsally. Legs 4 extend beyond the posterior end of the body by their two distal articles.

Female unknown.

Described from two specimens in the India Museum, Calcutta (no. $\frac{5992}{10}$ and no. ?), taken at Punkabani, Darjiling District, E. Himalayas (no host recorded).

This fine species—the largest male *Ixodes* known—may very likely prove to be the ♂ of *Ixodes acutitarsus* (Karsch) 1880, but it is unsafe at present to attribute it to that species. Its coxal armature is unique in this genus.

RHIPICEPHALUS BREVICEPS, n. sp.

(Fig. 3.)

Male unknown.

Female. Apparently about half gorged, 5 mm. long, long-oval.

Scutum. Long-oval (1·5 × 1·3 mm.), broadest at the eyes, which are large, black, well-defined, and rather nearer the posterior than the anterior border; dark and highly chitinised, with confluent punctations and rugae on the anterior half and small discrete punctations posteriorly. Cervical grooves rather faint, converging at first, then diverging, to disappear about the middle of the scutum. Lateral grooves curved, and reaching the posterior border, marking off a prominent external ridge finely punctate.

Capitulum. Excessively short; base three times as broad as long, with small shallow porose areas separated by more than their diameter. Palps no longer than in *Boophilus*.

Venter. Vulva very anterior (somewhat ill-defined in the specimen) between coxae I and II. Spiracles medium, short-comma-shaped.

Legs. Coxae normal; tarsi short, gibbous.

Described from a single specimen in the India Museum, Calcutta (no. $\frac{5954}{10}$), taken from *Erinaceus collaris* in Sind.

If this specimen is normal and mature, as would seem to be indicated by the highly chitinised scutum and the presence of porose areas, it is undoubtedly a new species. The vulva, however, is not well developed, and this gives rise to the suspicion that the capitulum also may be stunted. *R. sanguineus* generally occurs on this host, but the nature of the scutum renders it unlikely that this is an abnormally developed example of that species.

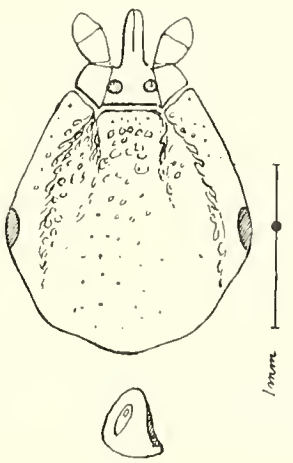

Fig. 3. *Rhipicephalus breviceps*, ♀. Capitulum, scutum and spiracle.

HAEMAPHYSALIS LONGIPALPIS, n. sp.

(Figs. 4 and 5.)

Male. 2 × 1·3 mm. (capitulum excluded), glossy, with numerous very small punctations, many of them confluent. Cervical grooves deep to receive the cornua, barely visible behind them; lateral grooves rather short and faint, including one festoon. Festoons long, with well-marked curved intervals.

Capitulum. Base sub-rectangular with rounded sides, broader than long, with cornua of almost equal length. Palps very long, three times as long as broad; article 2 very slightly protuberant laterally, unarmed, article 3 with a long, strong retrograde spine dorsally and ventrally; hypostome small, with numerous very small sharp teeth, apparently 6 | 6, considerably shorter than the palps.

Venter. Genital aperture between coxae II; spiracles subrectangular, more rounded anteriorly; anal groove rather ogival.

Legs. Coxae I with a rather long blunt spur, coxae II—IV unarmed. The second article of leg 1 has a distal retrograde spur both dorsally and ventrally, the former blunt, the latter shorter and more pointed. Tarsus 4 roundly tapering, pads large.

Female. Scutum nearly circular, 1·2 × 1·2 mm. Cervical grooves deep pits behind the cornua, followed, at a short interval, by broad, shallow, nearly parallel depressions extending slightly beyond the middle of the scutum. No lateral grooves. Numerous very small punctations.

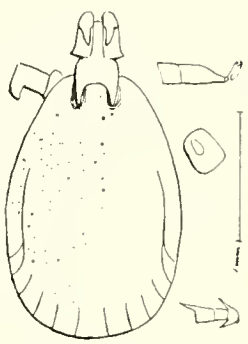

Fig. 4.

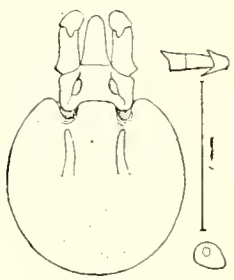

Fig. 5.

Fig. 4. *Haemaphysalis longipalpis*, ♂. Dorsal aspect, 4th tarsus, spiracle and profile of palp.

Fig. 5. *Haemaphysalis longipalpis*, ♀. Capitulum, scutum, profile of palp, and spiracle.

Capitulum. Base rectangular, twice as broad as long, porose areas much longer than broad, very far apart; cornua short and blunt. Palps very long, four times as long as broad, nearly cylindrical, armed as in the ♂, but with the dorsal retrograde spur of article 3 shorter. Hypostome 5 | 5, well covered with small teeth, spatulate.

Venter. Spiracle smaller than in the ♂ and more pointed dorsolaterally.

Legs. As in the ♂.

Described from 10 ♂ and 1 ♀ taken by Mr C. C. Dobell from *Tragulus meminna*, the Mouse Deer, at Colombo, Ceylon, 3, VIII, 1909.

This remarkable species, though undoubtedly a *Haemaphysalis*, has the abnormal character of palps much longer than broad. It belongs to the *H. bispinosa* group.

HAEMAPHYSALIS CUSPIDATA, n. sp.

(Figs. 6 and 7.)

Male. 1·8 × 1·1 mm. (capitulum exeluded).

Scutum. Fairly glossy, with numerous very small, shallow, diserete punetations. Cervieal grooves deep under the eornua, then faint and gently diverging; lateral grooves medium, faint, ineluding one festoon. Festoons long, with distinet brown intervals widening distally.

Capitulum. Base reetangular, broader than long, with straight sides and very long cornua, longer than the base (henee *cuspidata*). Palps of medium length, article 2 fairly salient laterally, unarmed, article 3 with long dorsal retrograde spine reaching to the posterior border of article 2, and almost equally long ventral spine, artiele 4 unusually long. Hypostome 4 | 4.

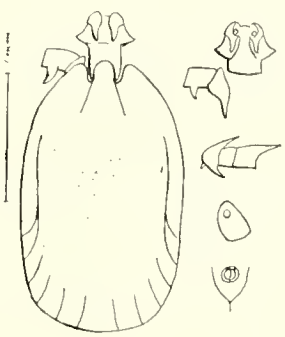

Fig. 6.

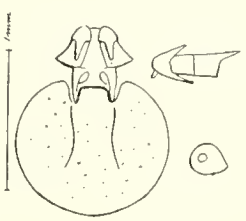

Fig. 7.

Fig. 6. *Haemaphysalis cuspidata*, ♂. Dorsal aspect, ventral aspect of capitulum and coxa I, profile of palp, spiracle, and anal groove.

Fig. 7. *Haemaphysalis cuspidata*, ♀. Capitulum and scutum, profile of palp, and spiracle.

Venter. Genital orifiee between coxae II; anal groove ogival; spiraele sub-triangular, with narrow rounded anterior border and nearly straight posterior border.

Legs. Coxae I with long narrow spur, eoxae II—IV unarmed, the troehanter of leg 1 with a strong rather sharp retrograde spur, both dorsally and ventrally; otherwise as in *H. longipalpis.*

Female. Body dark-brown, very punetate.

Scutum. Yellowish, nearly cireular, 1 mm. broad, slightly less in length. Numerous medium-sized punctations. Cervieal grooves rather long and slightly converging till they reach the middle of the scutum.

Capitulum. Base about twice as broad as long, with long cornua, but not so long as in the ♂; porose areas rather large, oval, converging in front, separated by more than their diameter. Palps with the same characteristics as those of the ♂, but the ventral spur on article 3 longer than the dorsal. Hypostome 4 | 4.

Venter. Spiracle nearly circular, but rather pointed dorso-laterally. Vulva between coxae II and III.

Legs. As in the ♂.

Nymph. Without dorsal spur in article 3 of palp; otherwise like the ♀ in such structures as are present.

Described from 30 ♂ taken (in company with *H. longipalpis*) by Mr C. C. Dobell from *Tragulus meminna* (the "Mouse Deer") at Colombo, Ceylon, 3, VIII, 1909, and 2 ♀ and numerous nymphs and larvae taken from *Paradoxurus niger* (Palm civet) at Colombo, and sent by Dr A. Willey, 16, II, 1910.

Though the sexes were taken from different hosts they correspond so precisely that there can be little doubt of their relation to each other. This species much resembles *H. longipalpis* in most respects, but it is easily distinguished by the capitulum.

HAEMAPHYSALIS LEACHI var. INDICA, n. var.

Differs from the type in the following respects:

Smaller, and shorter in proportion to its breadth. The external lateral contour of the palp is slightly concave, and its second article, though hollowed over the scapula, is without the characteristic dorsal and ventral retrograde processes. Cornua feeble. The scutum of the female is shorter and more accurately oval than in the type, where the postero-lateral borders are somewhat concave. Hypostome 4 | 4. Otherwise as in the type.

Male. 1·5 × ·8 mm. (without capitulum).

Female (unfed). 1·7 mm. long. Scutum ·8 × ·6 mm.

Described from several specimens taken from a jackal (*Canis aureus*) in the Museum Compound, Calcutta (tubes $\frac{5994}{10}$, $\frac{5995}{10}$).

The small size, less elongate form, and the absence of retrograde processes on the second article of the palps seemed, on first inspection, to indicate a distinct species, but the close agreement with the typical *H. leachi* in other respects prevents its recognition as anything more than a variety. It is perhaps worth noting that another African tick now widely spread in India—*Hyalomma aegyptium*—tends in that country to become smaller and less strongly characterised.

AMBLYOMMA ANNANDALEI, n. sp.

(Fig. 8.)

Male unknown.

Female. Inornate, chestnut brown.

Scutum. Cordiform, broader than long (2·5 × 3·3 mm.), antero-lateral sides convex, postero-lateral sides slightly concave, rather broadly rounded posteriorly. Cervical grooves deep, convex externally, followed by shallow divergent depressions. No lateral grooves. Eyes, large, flat, dark and inconspicuous. Punctations large and deep on the scapulae, very small between the cervical grooves, almost obsolete elsewhere.

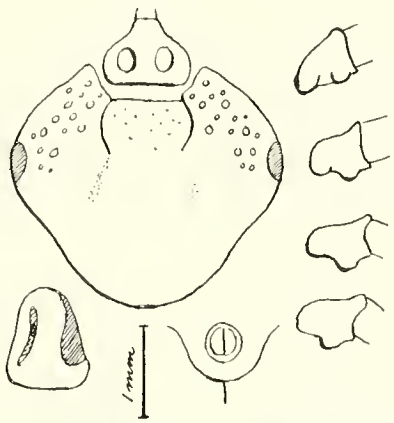

Fig. 8. *Amblyomma annandalei*, ♀. Capitulum and scutum, coxae, spiracle and anal groove.

Capitulum. Base sub-triangular with rounded sides; no cornua, but posterior border slightly concave; porose areas very deep, oval, longer than broad, separated by more than their diameter; palps of medium length, the second article rather more than twice the length of the third; hypostome? (absent in the specimen).

Venter. Vulva between coxae II; spiracle very large, sub-triangular with the apex anterior, the dotted area (white) bending sharply dorsally. Anal groove the arc of a circle.

Legs. Coxae I with two short, blunt, equal spurs; the inner spur diminishes progressively on coxae II and III and is absent on coxa IV. (The 4th tarsi are absent in the specimen.)

Described from a mutilated specimen in the India Museum, Calcutta (no $\frac{19}{1}$), labelled "on *Geoemyda spinosa*, India (Burma?)." Near *Amblyomma cyprium*.

APONOMMA GERVAISI, Lucas.

(Figs. 9 and 10.)

The examination of a large number of ticks taken from reptiles, especially in India, has convinced me that two forms which are at least entitled to rank as distinct varieties have been confounded under the name of *Aponomma gervaisi*. Both occur quite commonly, so that it is exceedingly unlikely that either is absent from any considerable collection, and they are sufficiently similar in facies, especially in ill-marked specimens, to make their confusion probable. On close examination, however, they seem to be quite separable, and I have come across no individuals which are not clearly attributable to one form or the other. Neumann's description[1] would apply fairly well to either form, so that it is not, perhaps, easy to decide which should be regarded as the type, but on the whole the choice must fall upon the smaller and more broad-oval form, especially as it is to this form that specimens in our possession, identified by Neumann as *A. gervaisi*, belong. For the other

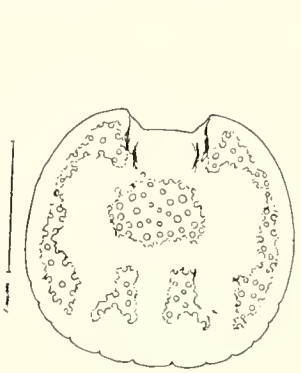

Fig. 9.

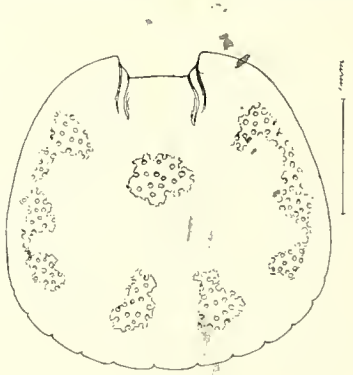

Fig. 10.

Fig. 9. *Aponomma gervaisi*, ♂. Diagram of contour and metallic patches.
Fig. 10. *Aponomma gervaisi*, var. *lucasi*, ♂. Diagram of contour and metallic patches.

and larger form I propose the name *A. gervaisi* var. *lucasi*. Before giving formal descriptions of the two forms it may be useful to point out the respects in which they chiefly differ. There will be no difficulty in distinguishing well-marked males, for though there is a general similarity in the distribution of the metallic blotches they differ markedly in one respect. In the type the elongated lateral blotches have an enlargement on a level with and external to the cervical

[1] *Rev. de la Famille des Ixodidés* (3), 1899, p. 182.

grooves. This enlargement is often detached, and is generally the most conspicuous part of the ornamentation, persisting when the rest is nearly obsolete. In *A. gervaisi* var. *lucasi* the lateral blotches barely reach as far forward as the cervical grooves, the scapulae being quite destitute of metallic markings. The respective females differ less obviously, but it is not difficult to distinguish them. The scutum of the type has larger punctations, larger metallic blotches, and is usually rounded posteriorly. That of *A. lucasi* is truncated or even slightly indented posteriorly and the punctations and metallic blotches are smaller. These and other differences may be exhibited thus in tabular form :

Male.

A. gervaisi.	*A. gervaisi* var. *lucasi.*
Broad-oval, broader than long, somewhat narrower in front, rounded posteriorly.	About as broad as long, the broadest part more posterior. Posterior border nearly straight.
Punctations normally large, often reducing the metallic blotches to mere reticulations.	Punctations small.
Anterior portion of lateral blotches on a level with cervical grooves.	No scapular metallic markings.
Basis capituli sub-pentagonal and without cornua.	*Basis capituli* more triangular, and with slight cornua.
Coxae I with two very slight dark points near together[1].	Coxae I more distinctly bifid, the points being stronger and more separate.

Female.

Scutum with large punctations and large metallic blotches; rounded posteriorly.	Scutum with smaller punctations and blotches; often truncate or slightly indented posteriorly.
Basis capituli sub-pentagonal, without cornua. Porose areas rather large and near together.	*Basis capituli* more triangular and with slight cornua. Porose areas smaller and further apart.

APONOMMA GERVAISI (Lucas).

Ixodes gervaisi, Lucas, *Bull. Soc. ent. Fr.* v. p. xcix, 1847.
Amblyomma arcanum, Karsch.
Ophiodes ophiophilus, Murray.
Ophiodes gervaisi, Murray.
Ixodes varanensis, Supino.

[1] Neumann (*loc. cit.*) says "une seule épine, courte, à toutes les hanches," but both forms show faint traces of two dark points on coxa I.

Male. Body rather broader than long (2 × 2·2 mm.), nearly round but somewhat narrower in front.

Scutum. Covering the whole dorsum, pitted with punctations which are somewhat variable but many of which are typically large and deep; a large part covered by metallic-green blotches, normally five in number, but frequently seven on account of the disintegration of the lateral blotches. A large median blotch, two sub-triangular posterior blotches, and two elongate lateral blotches with irregular internal contour, their dilated anterior portions on a level with, and external to, the cervical grooves, and often detached. Cervical grooves deep, convex externally. Festoons short, intervals distinct. No lateral grooves.

Capitulum. Long, base sub-pentagonal with rounded sides and straight posterior border, without cornua. Hypostome 3 | 3.

Venter. Spiracle large, about twice as long as broad, comma-shaped, with sharp dorsal curve.

Legs. Coxae I faintly bidentate, the two dark points near together; coxae II—IV with a single short internal spur. Tarsus 4 rather sharply prominent dorsally in the middle of the distal false articulation.

Female. *Scutum* cordiform, broader than long (1·3 × 1·7 mm.), rounded posteriorly. Punctations unequal, some being normally large and deep. Three large metallic-green blotches.

Capitulum. As in the ♂, but with rather large porose areas separated by less than their diameter.

Venter. Spiracles broader and shorter than in the ♂, the granulated area broad anteriorly and then proceeding almost at right angles dorso-laterally and terminating in a sharp dorsal curve.

Legs. As in the ♂.

APONOMMA GERVAISI var. LUCASI, n. var.

? *A. gervaisi* of various authors.

Male. Distinctly larger than the type. Body about as broad as long (2·7 × 2·7 mm.), not so round as *A. gervaisi*, but broadest more posteriorly, the posterior border being nearly straight.

Scutum. Covering the whole dorsum, with numerous rather small punctations. Five metallic-green blotches; a median blotch smaller than that of the type, two sub-triangular posterior blotches, and two elongate lateral blotches, less irregular than those of the type and

not attaining the scapulae. Cervical grooves as in *A. gervaisi*; lateral grooves absent.

Capitulum. Long; base more triangular, with slight cornua, which are dark and rounded.

Venter. Spiracles large, three times as long as broad, with sharp dorsal curve.

Legs. Coxa I more distinctly bidentate than in the type, with the points more separate. Tarsus 4 more roundly humped.

Female. *Scutum* cordiform, generally truncated or slightly indented posteriorly. Punctations rather small. The three metallic-green patches smaller than in *A. gervaisi*, especially the posterior patch.

Capitulum. Shaped as in the male, the porose areas small or moderate, and separated by their diameter.

Venter. Spiracles shorter than in the ♂ but of similar pattern, and differing similarly from those of the type.

Legs. As in the ♂.

NEW SPECIES OF TICKS (*IXODES, AMBLYOMMA, RHIPICEPHALUS*).

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Fellow of Magdalene College, Quick Professor of Biology, Cambridge.

(With 7 Text-Figures.)

The following descriptions relate to a new species of *Ixodes*, to a new variety of *Ixodes loricatus*, to new species of *Amblyomma* and *Rhipicephalus*:

Ixodes caledonicus n. sp. (♀, 0 and larva), from Scotland. (961, 1142, 1200.)[1]
Ixodes loricatus var. *spinosus* n. var. (♀), from Mexico. (647.)
Amblyomma v-*notatum* n. sp. (♀), from Manãos, Brazil. (1149.)
Rhipicephalus simpsoni n. sp. (♂, ♀), from Oshogbo, West Africa. (1214.)
(41 *b*, Ent. Res. Committee.)

Ixodes caledonicus n. sp.

Figs. 1—3.

Male: unknown.

Female (gorged): body[2] 7·8 × 4 mm., oblong, with sides almost parallel, posterior border broadly rounded, covered with a few short white hairs. *Scutum* (1·3 × 1 mm.): glossy, sub-oval, longer than broad, emargination slight, cervical grooves beginning as deep pits (giving the effect of sharp scapulae), then deep and distinct for about two-thirds the length, but slightly divergent and fading away toward the postero-

[1] These figures refer to numbers in our collection.

[2] The capitulum is *not* included in our measurements of body-length, because it may be retracted, protruded or inclined so as to render the measurement difficult. We measure from a line connectiug the scapular protrusions on the scutum (scapulae) to the posterior margin of the body. The greatest width only is given.

The *A* and *D* alongside the figures of spiracles denote their orientation ou the tick's body *A* pointing anteriorly, *D* pointing dorsally.

lateral borders; no lateral grooves; very fine, uniform punctations, except for a few larger ones along the anterior and antero-lateral borders; a few very small, short hairs. *Capitulum*: base sub-rectangular, broader than long, with slightly concave raised and trenchant dorsal ridge continuous with the slight (trenchant) cornua; porose areas not depressed, large, ovoid, almost confluent. Palps short, far apart basally, converging and rounded distally, with thick internal border and broadly rounded tips, articulations between articles 2 and 3 obsolete; thumb-like

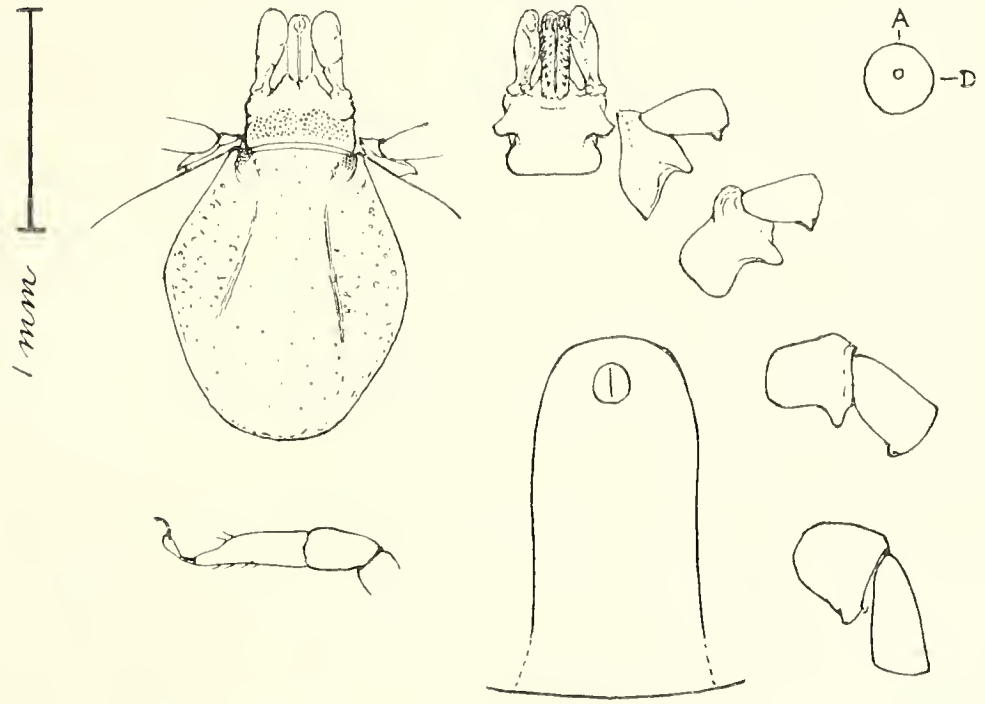

Fig. 1. *Ixodes caledonicus* ♀: capitulum and scutum, capitulum in ventral aspect with coxae, anal groove, tarsus 4 and spiracle. F. M. H. del.

in profile; ventral surface of basis capituli flattened, pentagonal, blunt auriculae protruding laterally; palpal article 1 with ventral angle; hypostome inclined ventrally, rounded in front, dentition 2|2, with 8—9 blunt teeth per file, and a narrow unarmed median ridge. *Venter*: vulva slightly posterior to a line connecting the posterior borders of coxae II; genital grooves rounded in front, diverging slightly, then sub-parallel, finally diverging slightly to the posterior border; anal grooves rounding the anus anteriorly, then parallel, slightly divergent behind. Spiracle circular, macula median. *Legs*: coxa I visible dorsally, bidentate; with stout external spur and short internal spur; a stout external spur

on coxae II and III, smaller on coxa IV; trochanters I—III with short spur at postero-external angles; tarsus IV long, tapering obliquely at the distal third of its terminal portion; claws much longer than the pad.

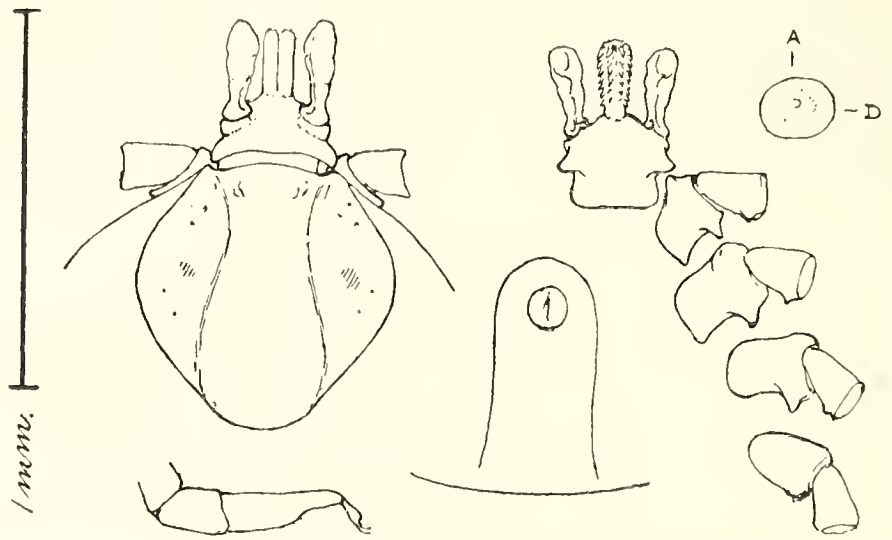

Fig. 2. *Ixodes caledonicus* ♀: capitulum and scutum with dorsal aspect of leg-pair I, ventral aspect of capitulum with coxae, tarsus 4, anal groove and spiracle. F. M. H. del.

Nymph: differs but slightly from the ♀: a few short (caducent) hairs on the scutum, longer hairs, in moderate numbers, on the body. *Scutum* as broad as long (0·7 × 0·7 mm.) with lateral angles rounded, with antero- and postero-lateral borders sub-rectilinear; cervical grooves attaining the posterior border. *Capitulum* with marked, trenchant cornua protruding outward and continuous with the turned upward dorsal ridge. Hypostome 2 | 2, with eight pointed teeth in the external files. *Venter*: spiracle bluntly oval. Otherwise resembling the ♀.

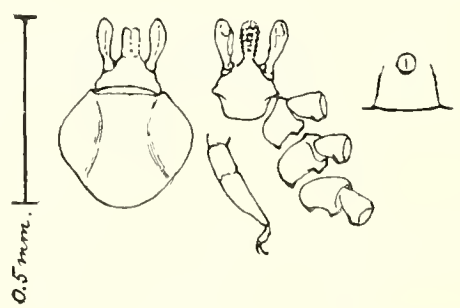

Fig. 3. *Ixodes caledonicus* larva: capitulum and scutum, ventral aspect of capitulum with coxae, tarsus 4, anal groove. F. M. H. del.

Larva (unfed): body 0·8 mm. long, resembles the ♀ and ♂ in its chief characters (coxae, trochanters, tarsi). *Scutum* more rounded (0·4 × 0·4 mm.) than in the ♂, with deep cervical grooves. *Capitulum*: hypostome 2|2, with six teeth in the external files.

Described from 1 ♀ (N. 961) found on rocks below a dove's nest, Fastcastle, Scotland, 6. IX. 1909, by Dr J. H. Ashworth (Edinburgh); 1 ♂ and 3 larvae (N. 1142) found on young domestic *pigeon*, from a dovecot at Duniface, Stirlingshire, Scotland, 18. IV. 1910, and 1 ♀ and 4 ♂'s (N. 1200) from the same source, 9. VIII. 1910, communicated by Mr William Evans (Edinburgh).

Ixodes loricatus var. **spinosus** n. var.

Fig. 4.

Male: unknown.

Female: differs from the type as follows:

It is larger and less hairy generally. The *scutum* (1·6 × 1·25 mm.) is broader and less punctate, there being about 20 rather coarse punctations situated in the median field. *Capitulum* broader (0·7 mm.) and relatively shorter, more massive, the ventral surface of the base

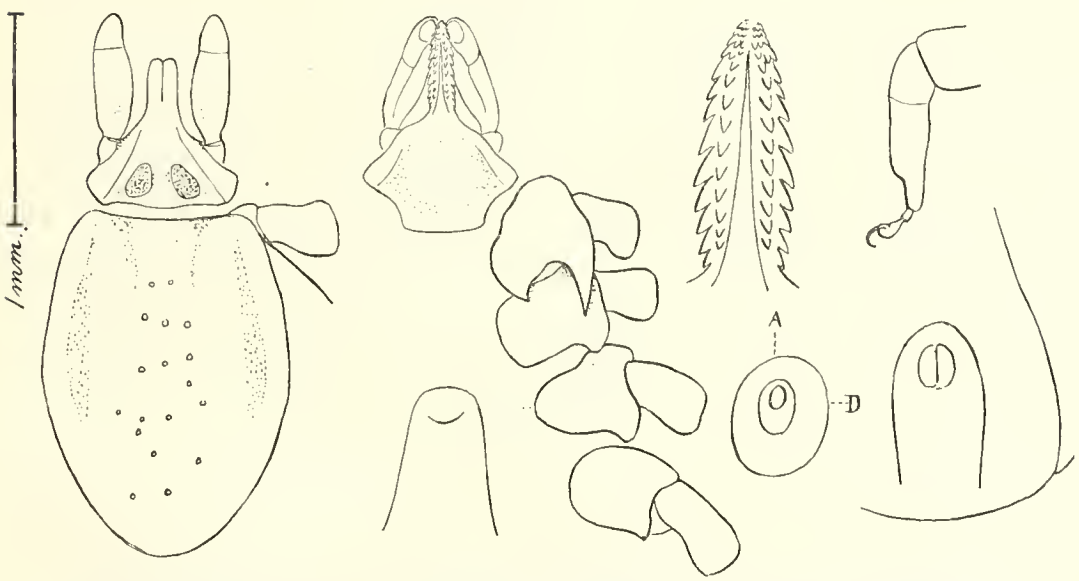

Fig. 4. *Ixodes loricatus* var. *spinosus* ♀: capitulum and scutum, ventral aspect of capitulum with coxae, vulva with commencing genital grooves; hypostome (highly magnified); spiracle, tarsus 4 and anal groove. G. H. F. N. del.

showing a broad U-shaped depression extending across the base posteriorly, the arms of the U reaching forward toward the base of the palps. *Venter*: spiracle larger (0·55 mm. l.) than coxa IV, and much nearer to the coxa. *Legs* more massive, *coxa I with large postero-external spur* prolonged in a point overlapping coxa II, with sharp internal angle; coxa II angular internally (rounded in the type). Tarsi tapering less gradually.

Described from (N. 647) 3 ♀'s, taken from a large *Opossum*, Tabasco de la Frontera, Mexico, in the month of May (*ex* Hon. N. C. Rothschild's collection).

Amblyomma v-notatum n. sp.

Fig. 5.

Male: unknown.

Female (unfed): 5 × 4 mm. *Body* contour ovoid, broadest at a point two-thirds along the body-length (measured from in front). Colour of chitinized parts generally maroon-brown. Hairs practically absent. Marginal groove and festoons sharply defined. *Scutum* (2·6 × 2·5 mm.) cordiform, deeply emarginate, scapulae rounded,

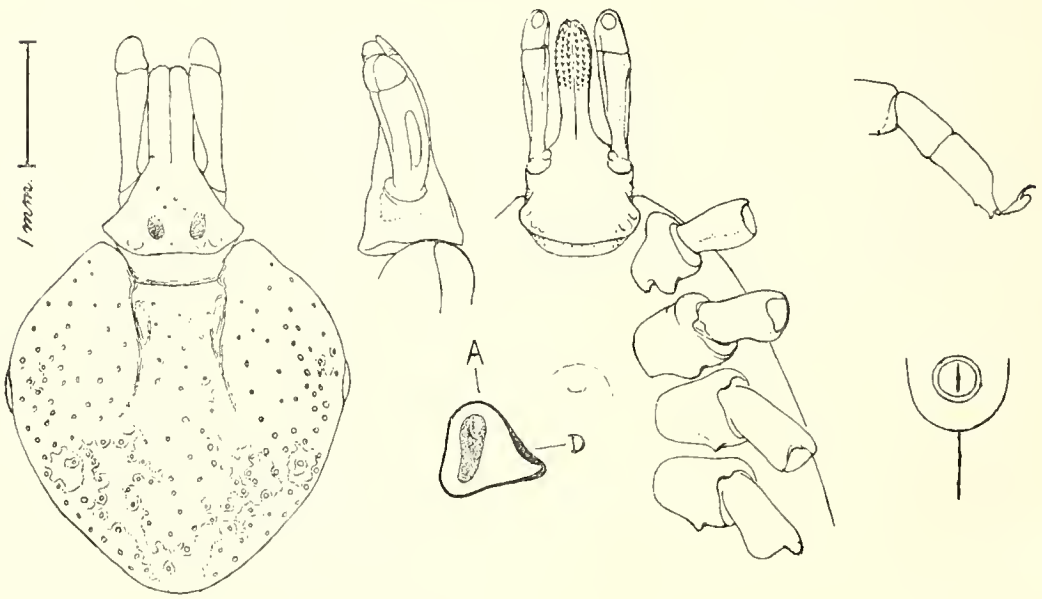

Fig. 5. *Amblyomma* v-*notatum* ♀ : capitulum and scutum (the ornate portions comprised posteriorly within the dotted lines), capitulum with scapula in profile (palp turned slightly outward), hence foreshortened; ventral aspect of capitulum with coxae and vulva; spiracle, tarsus 4 and anal groove. F. M. H. del.

posterior borders slightly convex, posterior angle somewhat ogival, uniformly coarsely punctate, with some larger punctations along the antero-lateral portions. Cervical grooves beginning as two oval pits posterior to the anterior border, but soon fading away to a point a little beyond half the scutum-length. Colour light in the median field, maroon-brown at the sides and along the postero-lateral borders, ornamented by an irregularly **V**-*shaped creamy patch* posteriorly with its apex directed backward and with two small rounded patches between the anterior points of the **V** and the lateral borders; slight traces of ornamentation anteriorly and at the sides. Eyes slightly in front of and midway along the scutum-length, elongate oval, colourless, but slightly protruding. *Capitulum* base roughly triangular, *with dorsal ridge continuous with protruding lateral ridges* (overlapping scutum) which in turn are continuous with lateral protrusions on the ventral surface. Porose areas deep, pyriform, with the points diverging somewhat anteriorly; some scattered punctations on the dorsal surface. Ventrally, the base is constricted posterior to the palps. Hypostome 3 | 3, toothed over half its length, with slight corona, 10 teeth on the external file, fewer on inner files. Palps, viewed dorsally, are broadest at the junction of articles 2 and 3; article 2 is two-and-one-half times as long as article 3. *Venter*: vulva facing the second intercoxal space; spiracle large, roughly triangular, anal groove, etc. normal (see Fig. 5). *Legs*: coxa I with rounded postero-external spur and small postero-internal tuberosity, small tuberosities near the postero-external angles of coxae II—IV. The movable joints normal, with whitish annulations at the distal ends of the articles. Tarsi tapering obliquely, with two slight spurs distally; claws long.

Described from (N. 1149) 4 ♀'s found on a sloth (*Bradypus tridactylus*), at Manãos, Brazil, by Mr T. P. Beddoes, 1903.

Rhipicephalus simpsoni n. sp.

Figs. 6—7.

Male: *Body* and *legs* reddish-brown; a few very small scattered hairs; the soft, pale, yellowish body extending (0·2 mm., or more) beyond the scutum and showing a rounded caudal protrusion in gorged specimens. Very variable in size. *Scutum* pear-shaped, broadest at a point three-fifths along the body-length (measured from in front) and anterior to the spiracles, deeply emarginate, with rounded scapulae; varies from

2·3 × 1·5 to 3·5 × 2·3 to 3·6 × 2·2 mm. in size; cervical grooves very short, only forming two deep oblong depressions, directed inward and backward; lateral grooves indicated by an irregular row of punctations ending on a line posterior to the eyes, beginning again, more outwardly, posterior to the eyes and merging rapidly into a well-marked groove, continued so as to include the second festoon; a few shallow punctations over the back, three longitudinal depressions on the posterior third,

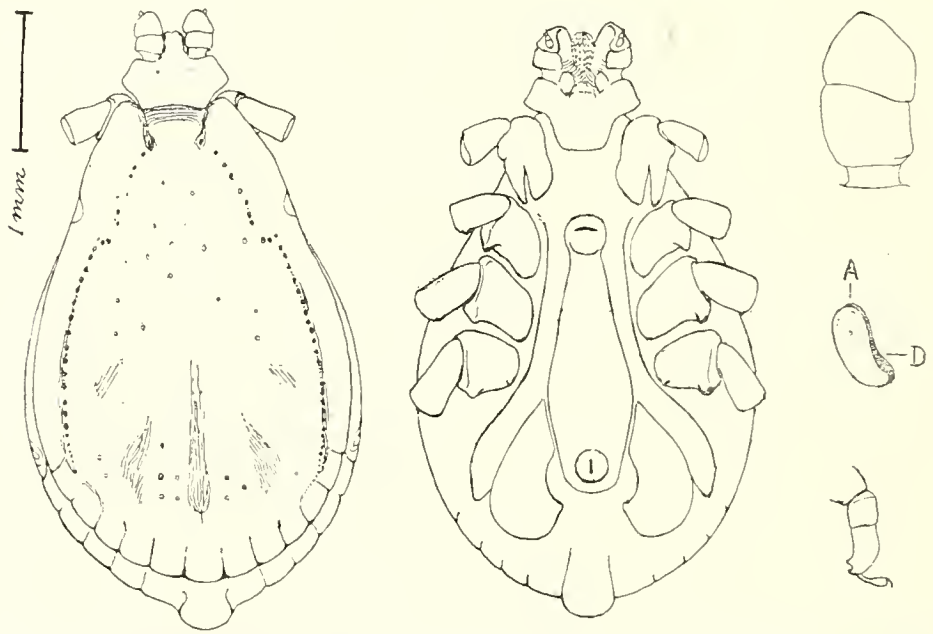

Fig. 6. *Rhipicephalus simpsoni* ♂: dorsum and venter, right palp highly magnified, spiracle and tarsus 4. F. M. H. del.

with more distinct punctations upon the intervening raised areas; festoons sharply defined, longer than broad, the median at times broader than the others, but usually not so. *Capitulum* base hexagonal, broader than long, narrow behind, with posterior and postero-lateral contours concave, antero-lateral margins straight; palps short, constricted basally, articles 2 and 3 of about equal length, with article 3 having a slight external angle; ventrally, article 1 bears an inwardly protruding flap, bearing some 6 forwardly curved hairs, article 2 bears 4—5 similar hairs along its internal border. Hypostome 3 | 3, with 8 teeth on the external file and a corona. *Venter*: sexual aperture facing coxa II; anus midway along the length of the broadly sickle-shaped adanal plates, whose incurved points face each other about two-thirds along their

length; accessory plates only slightly chitinized at their rounded tips; spiracles elongate comma-shaped. *Legs*: with coxa I showing slightly dorsally, bifid, with broad internal and slightly longer and narrow external spur; coxae II—IV with short, stout spur at the postero-external angle and (in some specimens) a small point at the postero-internal angle of coxa IV; tarsi very short and broad, with two small terminal spurs; claws slightly longer than the pads.

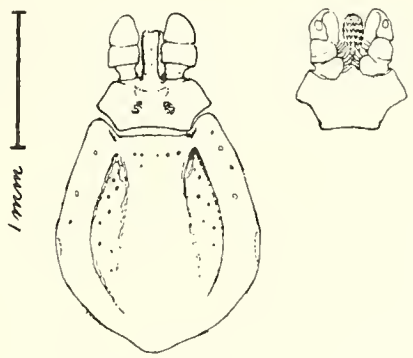

Fig. 7. *Rhipicephalus simpsoni* ♀: capitulum and scutum, ventral aspect of capitulum. F. M. H. del.

Female: resembles the ♂; *unfed*, the body varies in size from 2·5 × 1·5 to 3·75 × 2·5 mm., averaging about 3·4 × 2 mm.; marginal grooves, festoons, and few hairs present. *Scutum* longer than broad, varies in size from 1·4 × 1·2 to 1·8 × 1·65 mm., averaging about 1·7 × 1·6 mm.; scapulae rounded, deeply emarginate, antero-lateral contour but slightly convex back to and including the eye, posterior to which the contour is rounded and sinuous with a slight median protrusion. Cervical and lateral grooves starting together in a deep pointed pit, the cervicals distinct for half the scutum-length, then fading in a somewhat fusiform concavity lying between the median field and the raised lateral border, which is bounded internally by the lateral groove, the latter disappears near the posterior border; a few scattered punctations, some coarser ones accentuating the lateral groove; eyes pale, flat. *Capitulum* resembles that of the ♂, but with broader base; cornua faintly marked; porose areas small, ovoid, directed obliquely forward and inward, the interval equal to twice their width; palps longer than in the ♂. *Venter*: vulva facing coxa II; spiracle shorter and broader than in the ♂; festoons distinct. *Legs*, etc. resembling those of the ♂, movable articles slenderer.

Described from 5 ♂'s and 11 ♀'s found on a *large rodent*, Oshogbo, S. Nigeria, W. Africa, 28. II. 1910, J. J. Simpson (N. 1214). (41 *b*, Entomological Research Committee for Tropical Africa.)

We at first referred the specimens to *Rhipicephalus falcatus* Neumann, 1908[1], but on examining the types in the British Museum, and after consulting Professor L. G. Neumann, we have decided to accord them specific rank. In *Rhip. falcatus* ♂ the colour is blackish, the punctations numerous, the body and adanal shields narrower; in the ♀ the scutum is as long as broad (2 mm.), the punctations numerous. Owing however to the great range of variability which my colleague Mr Warburton and myself have observed in different species of *Rhipicephalus*, it is quite possible that some of the differences which we now regard as specific may ultimately prove to be merely varietal.

I am greatly indebted to Mr F. M. Howlett, B.A., Second Imperial Entomologist, Agricultural Research Institute, Pusa, Bengal, India, for very kindly executing, during his visit to Cambridge, the careful drawings (Fig. 4 excepted) illustrating this paper. All of the drawings were made from opaque specimens, preserved in alcohol, with the aid of a camera-lucida. I desire also to express my thanks to the Entomological Research Committee for Tropical Africa (Colonial Office), to the Hon. N. C. Rothschild, and to Messrs J. H. Ashworth, T. P. Beddoes and W. Evans for kindly placing the specimens here described at our disposal.

The expenses of these investigations on ticks are being partly defrayed through the aid of a grant from the Government Grant Committee of the Royal Society.

[1] Neumann, L. G. (VII. 1908). Notes sur les Ixodidés. *Notes from the Leyden Museum*, xxx. p. 77, Fig. 4. (*Rhip. falcatus*.)

SOME NEW INTERNAL PARASITES OF THE CALIFORNIA GROUND SQUIRREL (*OTOSPERMOPHILUS BEECHEYI*).

By CREIGHTON WELLMAN,
Oakland, California,
and WM. B. WHERRY,
Cincinnati, Ohio, U.S.A.

(With 10 Text-Figures.)

While examining last year some California ground squirrels, we found among this rodent's endoparasites the following protozoa, worm and mite which appear to be new. Ordinarily the mere recording of new species of the parasites of wild animals is of little interest to physicians, but since the rôle played by *O. beecheyi* in the maintenance of plague on our Pacific Coast was definitely established (*vide* Wherry, *Journ. Infect. Dis.* 1908, v. 485)[1] all medical men are naturally interested in the diseases which affect this rodent. Therefore the following brief descriptions of the parasites we have found are offered.

Protozoa.

Genus *Leucocytozoon* Danilewsky.

Leucocytozoon citellicola sp. nov.

Forms corresponding to trophozoits were present in considerable numbers in smears from the lungs, spleen, liver and inguinal glands. They were found almost entirely within the polymorphonuclear and

[1] See *Parasitology*, vol. II. p. 297 (*re* fleas on rodents in California); *Journ. of Hygiene*, vol. IX. p. 1 (anti-plague measures in San Francisco); also *Journ. of Hygiene*, vol. X. No. 4 (*re* plague among ground squirrels in America).

transitional leucocytes (Figs. 1 and 2). Occasionally they were extracellular (Fig. 3). They were best demonstrated with carbol thionin as they were surrounded by a capsular substance which usually resisted Wright's or Jenner's stains. Prolonged staining with Giemsa gave good results. In form they are elongated ovoid, sometimes slightly curved, with a rather large nucleus which stains uniformly and deeply purple with Giemsa after methyl alcohol fixation (Fig. 3), but appears reticulated or granular after heat and carbol thionin (Figs. 1 and 2). The

Fig. 1.

Fig. 2.

Fig. 3.

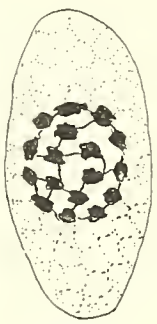

Fig. 4.

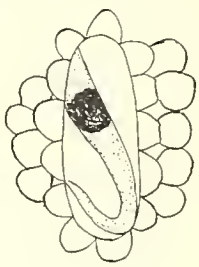

Fig. 5.

nucleus is often eccentric. The protoplasm is slightly reticulated and filled with basophilic granules after heat and carbol thionin and shows fine scattered reddish granules after Giemsa (Fig. 3). Measurements gave slight variations, the average being about 16 $\mu \times 6\ \mu$. A few larger forms were seen in smears from the lungs of squirrel 2. These were extracellular, non-encapsulated, 20 $\mu \times 10\ \mu$, oval with reticulated protoplasm and nucleus composed of isolated masses of chromatin (Fig. 4).

Type in the collection of Wm. B. Wherry.

Host. This *Leucocytozoon* was found in two ground squirrels collected in July and August 1909; one of these was also infected with a trypanosome.

Squirrel 1. Half-grown female; shot in the Berkeley Hills; appeared normal upon dissection with the exception of a slight congestion of the inguinal glands. Leucocytozoa were found, chiefly within the polymorphonuclear and transitional cells in smears from the lung, liver, spleen and inguinal glands.

Squirrel 2. Male, about one-third grown; shot in the Piedmont Hills. It appeared to be very ill and made no effort to escape from the hunter. Post-mortem the organs showed no noteworthy changes, excepting irregular, scattered areas of congestion in the lungs. Smears from the lungs showed no bacteria, but numerous motile trypanosomes were

seen. Leucocytozoa resembling those in squirrel 1 were found in smears from the lungs, spleen and liver.

Remarks. As far as we know the recent work of Miller (*Bull.* 46, *Hyg. Lab. U.S. Pub. Health and Mar. Hosp. Serv.* 1908) on *Hepatozoon perniciosum* of the white rat has not yet been confirmed and as we cannot say whether or not schizogony takes place in the liver of the ground squirrel we have retained the term *Leucocytozoon.* (See also Patton, *Parasitology,* 1909, II. 144.) No evidence of schizogony was found in the liver smears and the only body resembling a vermicule was found in a spleen smear (Fig. 5). It might be mentioned, since there is a communication between rats and ground squirrels, that this *Leucocytozoon* is somewhat larger and differs in detail, *e.g.* nuclear structure, from a similar parasite which is commonly found in the Norway rat (*M. norvegicus*) on the Pacific Coast. This parasite of the rat corresponds in size and nuclear structure with *H. perniciosum* Miller.

Genus *Trypanozoon* Lühe.

Trypanosoma Auctt.

Trypanozoon otospermophili sp. nov.

These parasites in squirrel 2 were actively motile and superficially resembled *T. lewisi.* After methyl alcohol and Giemsa the total length was 29·5 μ. From the pointed posterior end to the kinetonucleus 1·9 μ; kinetonucleus 0·6 μ; from the kinetonucleus to the posterior end of the nucleus 9 μ; nucleus 1·8 μ; from the nucleus to the end of the flagellum 16 μ; greatest breadth 1·5 μ (Fig. 6).

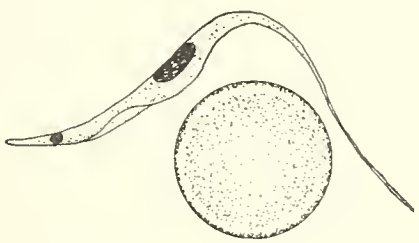

Fig. 6.

Type in the collection of Wm. B. Wherry.

Host. California ground squirrel (squirrel 2).

Remarks. Two young white rats and a guinea-pig free from trypanosomiasis were injected intraperitoneally with a physiological salt solution emulsion of the lung of squirrel 2. Their blood was examined

for trypanosomes every other day during the month of August with negative results. The white rats died on Aug. 28 and 30, but no cause of death was discovered. The ease with which rats are infected with *T. lewisi* suggests that our *Trypanozoon* is different.

Cestoda.

Collective Genus *Cystocercus.*

Cystocercus portolae sp. nov.

When fully extended the worm is somewhat pyriform (Fig. 7), the small end being the head. Neck very short, rostellum distinct, armed with two rows (which appear sometimes like one row very irregularly placed) of typical rose thorn hooks (Figs. 8 and 9). Suckers round.

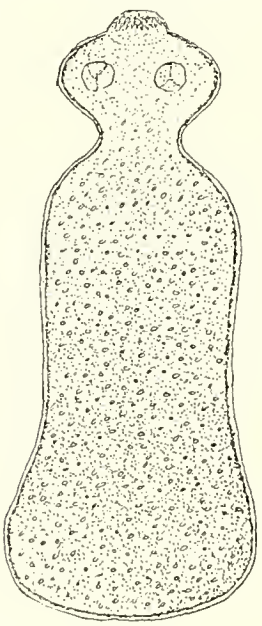

Fig. 7.

Fig. 8.

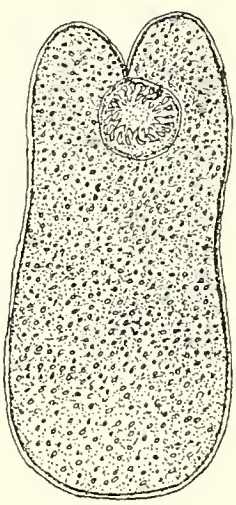

Fig. 9.

Epidermis transparent, a dark granular layer immediately beneath it, body tissue apparently homogeneous, granular, containing large calcareous corpuscles. Measurements of a fully extended specimen as follows: Total length 1·2 mm., breadth at widest part 0·406 mm., diameter of suckers 0·084 mm., width of rostellum 0·343 mm., length of rostellum 0·28 mm., width of neck 0·21 mm., average length of long hooks 0·028 mm., average width of long hooks 0·018 mm., average length of short hooks 0·019 mm., average width of short hooks 0·013 mm.

Type in collection of Creighton Wellman.

Host. *O. beecheyi* in small cysts in the liver substance.

Remarks. This cystocercus comes nearest to *C. longicollis* Rud., but is easily told from it by the fact that in *longicollis* the neck is longer than the body and the body darker than the neck, which features do not hold for our species. The character of the calcareous granules is also a differential point and according to Dujardin (*Hist. Nat. d. Helm.* 1845) the hooks in *longicollis* are different from those of our worm (Fig. 8).

C. longicollis is said to occur in moles and voles, and the adult (*Taenia crassiceps*, Zeder) to infest foxes. It is not improbable, therefore, as Dr Ransom, of Washington, first suggested to one of us, that the adult form of our worm will be found in coyotes or some such animal.

Acarina.

Genus *Cytoleichus* Mégnin.

Cytodites Mégnin.

Cytolichus Auctt.

Cytoleichus banksi sp. nov.

Resembles in its general features *C. nudus*, Vizioli. Body rounded, broadly oval, whitish, almost glabrous, not striated, but with very fine irregular, faintly marked ridges on the epidermis. Haustellum *shorter, broader and more truncate* than in *nudus*, viz. broadly conical, without cheeks. Legs strong, narrowly conical, composed of five articles and terminating in a non-armed pulvillus on a nearly transparent pedicle (Fig. 10) and a long, transparent seta. Sexual dimorphism not well marked: in the ♀♀ a genital pore (vulva or tocostome) may be made out in the median line between the next to last pair of legs. Average length 0·2 mm., average width 0·15 mm.

Type in the collection of Creighton Wellman.

Host. *O. beecheyi* (lungs).

Remarks. This species, the type of which was submitted to Mr Nathan Banks the eminent arachnologist and pronounced by him to be new, we place in the Cytoleichidae on account of the longitudinal genital aperture of the ♀. The specimens were found July 8, 1909, occurring in large numbers in small grayish, rounded, slightly raised tubercles on the lungs of two specimens of ground squirrels. The tubercles also exist in the deeper lung tissue. One mite was found in each tubercle

examined. The presence of mites belonging to this family (the "Sarcoptides cysticoles" of Mégnin) in the lungs and other tissues of birds has been known since Gerlach's observations in 1859. An especial medical interest has been added to the family by the finding by Castellani of one species (*C. sarcoptoides* Cast.) in the omentum of a negro (*Centralbl. f. Bakt.* Abt. 1. XLIII. p. 372).

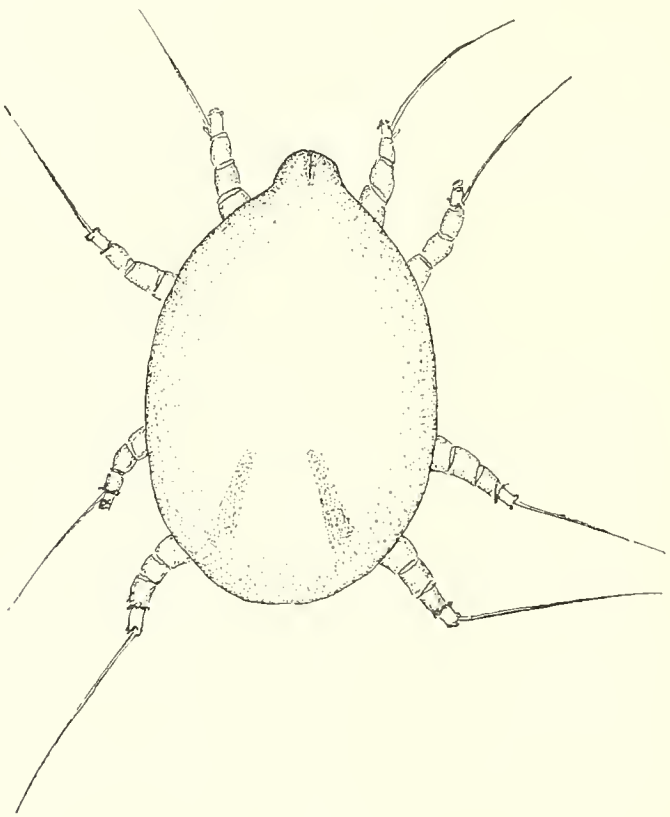

Fig. 10.

We have not included in this paper the ectoparasites found by us on *O. beecheyi.* These consisted of different fleas, ticks, etc. Among the latter were two very interesting species, one being the common *Dermacentor occidentalis* Neum. and the other a new species *Ixodes aequalis* Banks (*vide Entomological News,* Nov. 1909, p. 276). It is to be hoped that the ground squirrel examined by us will be studied as carefully as the rat has been and to such a study we contribute the facts contained in this paper.

DEGENERATION PHENOMENA OF *TRYPANOSOMA GAMBIENSE.*

By EDWARD HINDLE, Ph.D., A.R.C.S.,
Beit Memorial Research Fellow.

(*From the Quick Laboratory, Cambridge.*)

(With Plate XXX.)

In spite of the numerous papers that have been devoted to a consideration of the morphology of *Trypanosoma gambiense*, our knowledge of the life-history of this parasite, both in the vertebrate and invertebrate hosts, is very incomplete. One of the reasons for this is undoubtedly the practical difficulty of studying the parasite in its natural surroundings, and in its normal vertebrate host, man, and up to the present most workers on this subject have confined their attention to the morphology of this trypanosome when under somewhat unnatural conditions, for none of the ordinary experimental animals (rats, guinea-pigs, etc.) in which it is studied, are known to be susceptible to natural infection. Therefore, any results obtained from such studies of the parasite must be regarded as indicating only what *probably* takes place under natural conditions, and, as such, remain *sub judice* until confirmed by observations in the normal hosts.

Although white rats, the animals used for the following observations, are not known to be susceptible to natural infection with *T. gambiense*, yet it is evident, from the well-marked periodicity in the number of parasites in the peripheral circulation, that some kind of life-cycle takes place in this host. In attempting to follow this cycle it was found

impossible to distinguish between the normal trypanosomes and the large number of degenerating forms mixed up with them, and therefore, at Professor Nuttall's suggestion, an attempt has been made to trace some of the stages in the degeneration of this parasite.

In order to ensure the degeneration of all the trypanosomes in the blood, drug treatment was employed. Seven hours after a subcutaneous injection of 1 c.c. of a 2 % solution of arsenophenylglycin into a heavily infected rat (the blood containing 60—80 parasites to a microscopic field) no parasites could be observed in the peripheral circulation, and so by making a series of films at short intervals between the time of injection and seven hours later, the various stages in the degeneration of the trypanosomes could be observed. It was also found that *T. gambiense* degenerates very rapidly on being taken into the alimentary canal of *Ornithodoros moubata*, and a second series of films was prepared from the contents of the gut of a tick which had previously been fed upon an infected animal, but the degeneration forms observed do not differ in any marked degree from those occurring in the blood of a rat after drug treatment.

Technique.

In all cases rats were the experimental animals employed because of the large number of trypanosomes occurring in the peripheral circulation. When the number of parasites in the blood of the infected animal had reached as high as 60—80 to the microscopic field, an injection of 1 c.c. of a 2 % solution of arsenophenylglycin was administered subcutaneously, and films made from the blood of the rat at intervals of one hour. As a general rule the trypanosomes had disappeared from the circulation 6—7 hours after the injection and, therefore, films were only made during the first seven hours.

Both the "dry" and "wet" methods of fixation were employed. In the former case the films were fixed in absolute alcohol and then stained either with Giemsa or Azur, the latter giving the better results, especially for demonstrating the axial filament. In the second case, the films were fixed in strong Flemming's solution, and afterwards stained either with iron haematoxylin, or with safranin and methylene blue. (Salvin-Moore and Breinl, 1907.)

For the study of the granules and the general form of the trypanosomes dry films were found to be the most useful, but for the finer details the wet films were far superior.

Observations on the living parasites.

As in the case of *T. brucei* (Nuttall, 1910), the trypanosomes shewed increasingly active movements up to 1—1½ hrs. after the injection, but after this their motion gradually diminished until after 5 hrs. only the flagellum moved slowly. Shortly after treatment the trypanosomes appeared more granular and opaque, and the number of granules could be seen increasing, until finally the parasites broke up into fragments which were ingested by the leucocytes. The short stumpy forms are the last to disappear from the blood, and therefore the percentage of them gradually increases as the other forms die off.

The number of parasites in the peripheral circulation begins to diminish immediately after the injection of arsenophenylglycin, as is shewn in the following table.

Treated Rat A.

Time		Number of parasites
9.30 a.m.	... ...	80 per micr. field.
10.00 a.m.	Rat, weight 100 gms., inj. subcut. with 1 c.c. of 2 % sol. of arsenophenylglycin.	
10.30 a.m.	... ...	75 ,, ,,
11.30 a.m.	... ...	65 ,, ,,
12.50 a.m.	... ...	50 ,, ,,
1.30 p.m.	... ...	20 ,, ,,
2.30 p.m.	... ...	1 to 10 fields.
3.30 p.m.	... ...	1 to film.
4.30 p.m.	... ...	negative.

In this respect, therefore, the behaviour of the parasites after treatment with arsenophenylglycin differs from that after atoxyl, as in the latter case the number of trypanosomes in the peripheral circulation usually increases shortly after the injection and only subsequently diminishes. In both cases, however, the first effect of the drug is to cause an increased activity in the trypanosomes and afterwards granular degeneration. The number of leucocytes in the blood increases after an injection of arsenophenylglycin; the same phenomenon has also been observed after treatment with atoxyl.

Observations on the living parasites stained in vitro.

A certain number of observations were made on the living parasites stained with neutral-red. It was found most convenient simply to run under the edge of a coverslip preparation of the blood a drop of

a solution of the stain in saline, in the manner described by Policard (1910). The tropho-nucleus was only slightly coloured by this stain but the kineto-nucleus and granules were stained a brick-red colour, and, in most cases, the latter were arranged in either one or two longitudinal rows in the region of the body between the tropho-nucleus and the anterior extremity. In one or two cases the extrusion of a granule from the tropho-nucleus was observed. Because of the fact that these granules may be found in some trypanosomes even at an early stage of infection, Policard infers that they are not degeneration products; but from the fact that the percentage of trypanosomes containing them goes up from 5 %—10 %, before treatment, to almost 100 % after, when the parasites are certainly degenerating, it seems more reasonable to suppose that individual forms are dying off at all stages of infection and these contain granules.

Up to the present it has been found impossible to demonstrate the axial filament by the use of *intra vitam* stains.

Observations on the stained parasites.

Of the methods employed wet films stained with iron haematoxylin, and dry films stained either with Azur or Giemsa, were found to be the most useful. The first method stains both nuclei together with the karyosome and intra-nuclear granules, also the degenerating axial filament (figs. 13—14) and certain chromatic granules occurring between the two nuclei (fig. 9). The granules occurring in that part of the cell anterior to the tropho-nucleus are not stained by this method, and therefore it is possible to distinguish two kinds, for both varieties stain equally well with Giemsa or Azur, which, in addition, colour all the other structures enumerated above.

After staining with Giemsa both the intra- and extra-nuclear granules appear red, and in order to distinguish between them the films were afterwards treated with Gram's Iodine Solution (Swellengrebel, 1909). The films were first stained for $\frac{1}{2}$ hr. with Giemsa, then rinsed with water and treated with Gram's Iodine Solution for 1 min. and again washed in water. After this treatment the extra-nuclear granules of volutinose appeared black, sometimes with a reddish tinge; some of the granules within the tropho-nucleus appeared the same colour; the chromatin granules of the tropho-nucleus were greyish-brown; the kineto-nucleus appeared dark-red, and the plasma greyish-blue.

By this method it was thus possible to see that the extra-nuclear granules were first formed within the tropho-nucleus and subsequently extruded into the cytoplasm.

(*a*) *The trypanosomes before drug treatment.*

The study of the normal trypanosomes, both in dry films stained with Giemsa, and in wet films stained with iron haematoxylin, has revealed the presence of three distinct types, and thus we can confirm the observations of most investigators regarding the trimorphism of this parasite. The details of the origin and multiplication of these forms are reserved for a future publication, and in the present paper we shall merely consider the structure of the most common type of trypanosome occurring in the blood, viz., the indifferent form.

In this type the kineto-nucleus is a small oval body situated a short distance from the posterior extremity. The end-bead of the flagellum is in very close relation to the kineto-nucleus so that the two structures usually appear united. Typically the tropho-nucleus consists of a small intra-nuclear centrosome surrounded by a clear ovoid space containing nucleo-lymph, which is bounded on the outside by a delicate nuclear membrane. The chromatin is usually precipitated on the centrosome in the form of a dense karyosome, but trypanosomes may be found in which the chromatin is precipitated on the nuclear membrane. Most commonly the tropho-nucleus, in addition to the karyosome, contains one or more chromatic granules, which are very variable both in number and size, but are usually arranged in a longitudinal series.

In many of the trypanosomes, immediately in front of the kineto-nucleus, is a small vacuole, for which we propose the name "kineto-vacuole," from the anterior corner of which an achromatic axial filament runs down the centre of the body to near the anterior extremity. In many cases the axial filament arises from a small and rather indistinct granule on the edge of the kineto-vacuole, and it usually ends in another slight dilatation at its anterior end.

The axial filament was first noted in trypanosomes by M. Robertson (1906) in *T. brucei*, and has since been found in *T. gambiense*, and *T. equinum*, by Swellengrebel (1909). Whilst on the whole agreeing with the observations of these two authors, those recorded below seem to point to the fact that the axial filament may not pass through the tropho-nucleus, but to one side of it, and the appearance presented by some of the trypanosomes in Swellengrebel's figures may be due to the

filament drying down on to the tropho-nucleus and thus appearing to pass through it.

The axial filament does not seem to be constantly present and is usually very difficult to trace along its whole length, but from the arrangement of the granules in either one or two longitudinal rows, it seems possible that it may be present even when not visible. During certain kinds of degeneration this structure may become densely chromatic and is then very conspicuous (figs. 13—16), but as a rule it is almost achromatic.

With the exception of one at each end of the axial filament, it is not usual to find granules occurring in the cytoplasm of the trypanosomes before treatment. At times, however, a few granules of volutinose may be extruded from the tropho-nucleus, but they rapidly dissolve in the cytoplasm. They probably represent degeneration products of the chromatin which have been cast out by the nucleus, especially when under abnormal conditions.

Certain chromatic granules are also given off from the kineto-nucleus and pass forward towards the tropho-nucleus (figs. 2, 6). The nature of these granules is uncertain, but they are unquestionably different from the volutinose granules of the tropho-nucleus, as they stain with iron haematoxylin and are often present before treatment.

(*b*) *The trypanosomes after treatment with arsenophenylglycin.*

Shortly after the injection the most noticeable alteration in the appearance of the trypanosomes is the retraction of the posterior end, so that the kineto-nucleus appears at the extremity of the body, and in some cases even outside it (figs. 29, 34). This phenomenon has been described by Nuttall (1910) in the case of *T. brucei* after treatment with arsenophenylglycin, and is most clearly seen in dry films.

A differential count of the trypanosomes before and after treatment brought out the fact that whereas before the injection only 22 %[1] possessed blunt posterior extremities, one hour after 96 % shewed this rounded appearance.

The number of parasites containing granules, especially in the anterior region of the body, increases to nearly 100 % within two hours after treatment, their number gradually increasing until the parasite finally breaks up into fragments. As mentioned above, it is possible to

[1] The presence of the "stumpy forms" of *T. gambiense* makes this percentage higher than in the case of *T. brucei*.

distinguish two kinds of extra-nuclear granules, viz. chromatic, which are derived either from the kineto-nucleus or the axial filament, and granules of volutinose derived from the tropho-nucleus.

For the sake of convenience the latter will be considered first, for they are an essential feature in the degeneration of the parasites. These granules have been shewn by Swellengrebel (1909) to consist of a substance allied to volutin, for which he has proposed the name "volutinose."

The granules of volutinose appear bright red in dry films stained with Giemsa or Azur, and after treatment with Gram's Iodine solution appear almost black. They are not stained with iron-haematoxylin, which thus constitutes an easy mode of distinguishing them from the chromatic granules.

The volutinose is formed within the tropho-nucleus, probably as the result of chromatin degeneration, and by means of the above-described method (Gram's Iodine etc.) it is possible to distinguish them amongst the chromatin granules in the tropho-nucleus. They are usually extruded from the anterior end of the nuclear membrane (fig. 29) and pass forward towards the anterior extremity in a longitudinal row (figs. 32—34). During the last stages of degeneration, however, the granules are given off from all parts of the nucleus (fig. 35) and may become irregularly scattered throughout the cell (figs. 42, 43).

That these granules are merely the products of degeneration is most clearly demonstrated by comparing the percentage of trypanosomes containing them before and after treatment. In one case whereas before treatment only 5 % of the trypanosomes contained granules in the anterior region of the body, two hours after the injection 99·5 % contained them. The percentage containing these granules also rises as the parasites become more numerous and consequently greater numbers of them are degenerating.

The fact that the granules are almost invariably arranged in one or two longitudinal rows suggests that an axial filament may be present even when it is not visible. If one does not assume its presence, it is impossible to explain the fact that the granules are so often arranged in a longitudinal row extending from the tropho-nucleus to the anterior extremity, two rows being present when the axial filament has divided (figs. 37—39).

Further evidence in support of the view that the volutinose granules are arranged along an axial filament is afforded by the appearance of dividing forms. The axial filament usually divides at

the same time as the kineto-nucleus, and trypanosomes may be found containing a longitudinal row of granules all dividing at the same time to form two rows (figs. 31, 37—39). This appearance can only be explained on the assumption of the presence of an axial filament, which must usually be achromatic, as it is only occasionally seen.

In the later stages of degeneration when the axial filament has disintegrated, the granules appear irregularly scattered throughout the cytoplasm (figs. 42, 43), but they are always concentrated towards the anterior extremity (fig. 36). In fact, it seems to be characteristic of all the granules, whatever their origin, to move towards the anterior extremity of the body.

The size of the granules is somewhat variable, but as a rule they are large when first extruded (fig. 32), and subsequently diminish in size as they dissolve in the cytoplasm (fig. 33). When the amount of nuclear matter is small the whole of the tropho-nucleus may break down into volutinose granules which completely dissolve in the cytoplasm before the cell form is lost (fig. 44). Usually, however, the trypanosome breaks up into fragments whilst the cytoplasm is still loaded with granules and contains a distinct tropho-nucleus, which is often the last structure to disappear (figs. 24, 45). These free nuclei bear some resemblance to Salvin-Moore and Breinl's "latent bodies" (1907), but we have not found any evidence that they may be regarded as representing anything else but the products of degeneration.

The other kinds of granules, which, for the sake of convenience, have been grouped together under the name of chromatic granules, are easily distinguished from volutinose, as they stain with iron haematoxylin, as well as with Giemsa.

They have two distinct modes of origin, in the one case arising from the kineto-nucleus, whilst in the other they are derived wholly, or in part, from the breaking down of the axial filament. It is difficult to distinguish between them, however, as under certain conditions the chromatin from the kineto-nucleus seems to pass along the axial filament to form the so-called "black-line," and when this breaks up into granules they usually contain chromatin.

The particles of chromatin that are given off from the kineto-nucleus have been observed in various species of trypanosomes (e.g. Salvin-Moore and Breinl, 1908; Hindle, 1909), and Salvin-Moore and Breinl (1908), consider the passage of a chromatic body from the kineto-nucleus to the region of the tropho-nucleus to have considerable importance in the life history of *T. equiperdum*, regarding it as being homologous with the

development of the "black line" in *T. gambiense*. In this latter trypanosome, however, it is also possible to trace the passage of chromatin from the kineto-nucleus to the region of the tropho-nucleus (figs. 2, 6, 40), and it is rather difficult to understand why *T. gambiense* should require two methods of transferring chromatin from one nucleus to the other.

The nature of these granules is rather problematical, for although the percentage of trypanosomes containing them rose from 30 %, before treatment, to 60 % two hours after, yet this difference is not sufficiently well-marked to warrant the assumption that they are merely degeneration products.

As a rule these granules (or chromatic bodies) seem to be derived from the kineto-nucleus by means of an aberrant division, after which one of the daughter nuclei passes forward towards the tropho-nucleus without developing a flagellum. On its way it often divides (figs. 2, 6, 38), and these two granules persist for a long time in the region of the tropho-nucleus finally, however, dissolving in the cytoplasm. Occasionally a succession of these bodies is given off from the kineto-nucleus and forms a longitudinal row extending down the middle of the cell.

The degeneration of the axial filament and its associated structures is best considered at this point, together with the granules arising from them.

The kineto-vacuole, when present, begins to enlarge shortly after treatment, and in advanced stages of degeneration may become as large as the tropho-nucleus (figs. 40—52). Under certain conditions the vacuole becomes filled with chromatic substance, probably derived from the kineto-nucleus, and is then a very conspicuous feature of the cell (fig. 12).

As the axial filament degenerates it usually becomes chromatic (figs. 7—10), and then breaks up into a series of granules (figs. 8, 33) arranged in a longitudinal row.

Occasionally chromatic substance from the kineto-nucleus seems to flow round the kineto-vacuole and along the axial filament, which then stains black with iron haematoxylin. The passage of this densely chromatic substance along the achromatic axial filament gives the appearance of a black line growing down the middle of the cell, and Salvin-Moore and Breinl (1907) regarded it as being of considerable importance in the life-cycle of *T. gambiense*. They suppose that this "black line" formation represents a kind of autogamy, and figure stages shewing the growth of the black line from the kineto-nucleus to the region of the tropho-nucleus. But it will be seen from figs. 14, 15 that

this peculiar structure may extend to the anterior extremity of the cell, and there is no evidence to suggest that it ever becomes connected with the tropho-nucleus. On the contrary, the fact that it is only seen when numbers of the trypanosomes are degenerating, and the subsequent behaviour of the band, which gradually becomes less stainable (fig. 16) and breaks up into granules, clearly shew that it is merely one of the phenomena of degeneration. The "black line" was found to appear in some of the parasites 2—3 hours after an injection of arsenophenylglycin, even when no trace of it could be found in the trypanosomes immediately before treatment; and the fact that naturally it only occurs at the height of infection, when large numbers of degenerating forms are present, confirms the view that it is of no significance in the life history of *T. gambiense*, but is merely a degenerating axial filament containing chromatic substance. The granules which arise from the breaking down of the axial filament very rapidly dissolve in the cytoplasm and disappear.

In addition to the degeneration phenomena described above, after treatment one occasionally sees rounded up forms (fig. 25) which somewhat resemble those described by Salvin-Moore and Breinl (1907) in *T. gambiense* after treatment with atoxyl. There is no evidence, however, to support the idea that they represent resistant forms, for we have never found any trace of them except during the first few hours after the injection of the drug.

Summary of Results.

After treatment with arsenophenylglycin the number of trypanosomes in an infected animal immediately begins to decrease, and the parasites disappear from the peripheral circulation 6—7 hours after the time of injection, the short stumpy forms being the last to degenerate.

One of the first changes noticed in the trypanosomes after treatment is the rounding up of their posterior extremities caused by the retraction of the protoplasm behind the kineto-nucleus. Meanwhile the tropho-nucleus becomes filled with granules of volutinose, which are extruded into the cytoplasm and pass along the axial filament towards the anterior extremity of the body. In dividing forms the granules form two longitudinal rows, being distributed along the two axial filaments. Finally, the granules lose their regular arrangement and become scattered throughout the cytoplasm, being more abundant, however, at the anterior end.

The kineto-vacuole, when present, enlarges considerably and may become filled with chromatic substance derived from the kineto-nucleus.

The axial filament begins to degenerate soon after treatment, often breaking up into a row of granules. Occasionally, however, chromatic substance from the kineto-nucleus passes along the axial filament, and it then appears as a thick black band extending along the middle of the cell eventually to the anterior extremity. Subsequently it breaks up into granules which dissolve in the cytoplasm of the cell.

Chromatic bodies are given off from the kineto-nucleus both before and after treatment and do not seem to be a characteristic feature of degeneration.

Sometimes the tropho-nucleus completely disappears before the cytoplasm breaks up into fragments, but at other times it preserves its form long after all other parts of the trypanosome, with the exception of the flagellum, have disappeared. These free nuclei resemble the "latent bodies" described by Salvin-Moore and Breinl, but as they, along with the fragments of cytoplasm, granules etc., are ingested by the leucocytes, it seems possible that they are merely one of the results of degeneration and not part of the life-cycle of this parasite.

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EXPLANATION OF PLATE XXX.

All the figures are drawn to a magnification of 2400 diameters with the exception of Figs. 17—23 which are magnified 4000 diameters.

Figs. 1—25. *Trypanosoma gambiense*, drawn from films stained with iron-haematoxylin.

Fig. 1. Indifferent form, 1 hr. after treatment, shewing rounded posterior extremity.

Fig. 2. Similar form shewing a small kineto-vacuole and chromatic granules between the two nuclei.

Fig. 3. Trypanosome, 1 hr. after treatment, shewing a trace of the axial filament between the kineto-vacuole and tropho-nucleus, the latter containing granules of volutinose.

Fig. 4. Ordinary dividing form, 1 hr. after treatment.

Fig. 5. Dividing trypanosome, 2 hrs. after treatment, shewing a black line extending down from the kineto-vacuole.

Fig. 6. Trypanosome, 1 hr. after treatment, shewing a dividing granule between the two nuclei.

Fig. 7. Trypanosome, 2 hrs. after treatment, containing a spiral band of chromatin in place of the tropho-nucleus, and partly developed axial filament.

Figs. 8—10. Various Degeneration stages of the axial filament. (2 hrs. after treatment.)

Fig. 11. Small form, 3 hrs. after treatment, containing a well-developed "black line" extending to the anterior extremity.

Fig. 12. Advanced stage of degeneration, 3 hrs. after treatment. The kineto-vacuole is filled with chromatic substance and the tropho-nucleus has broken up into fragments.

Fig. 13. Trypanosome, 2 hrs. after treatment, shewing a deeply stained axial filament (black line) ending about the region of the tropho-nucleus.

Fig. 14. Drawn from the same film as the preceding figure.

Fig. 15. Large dividing (?) form, 3 hrs. after treatment, with a well developed axial filament arising from a large kineto-vacuole.

Fig. 16. Male form, 3 hrs. after treatment.

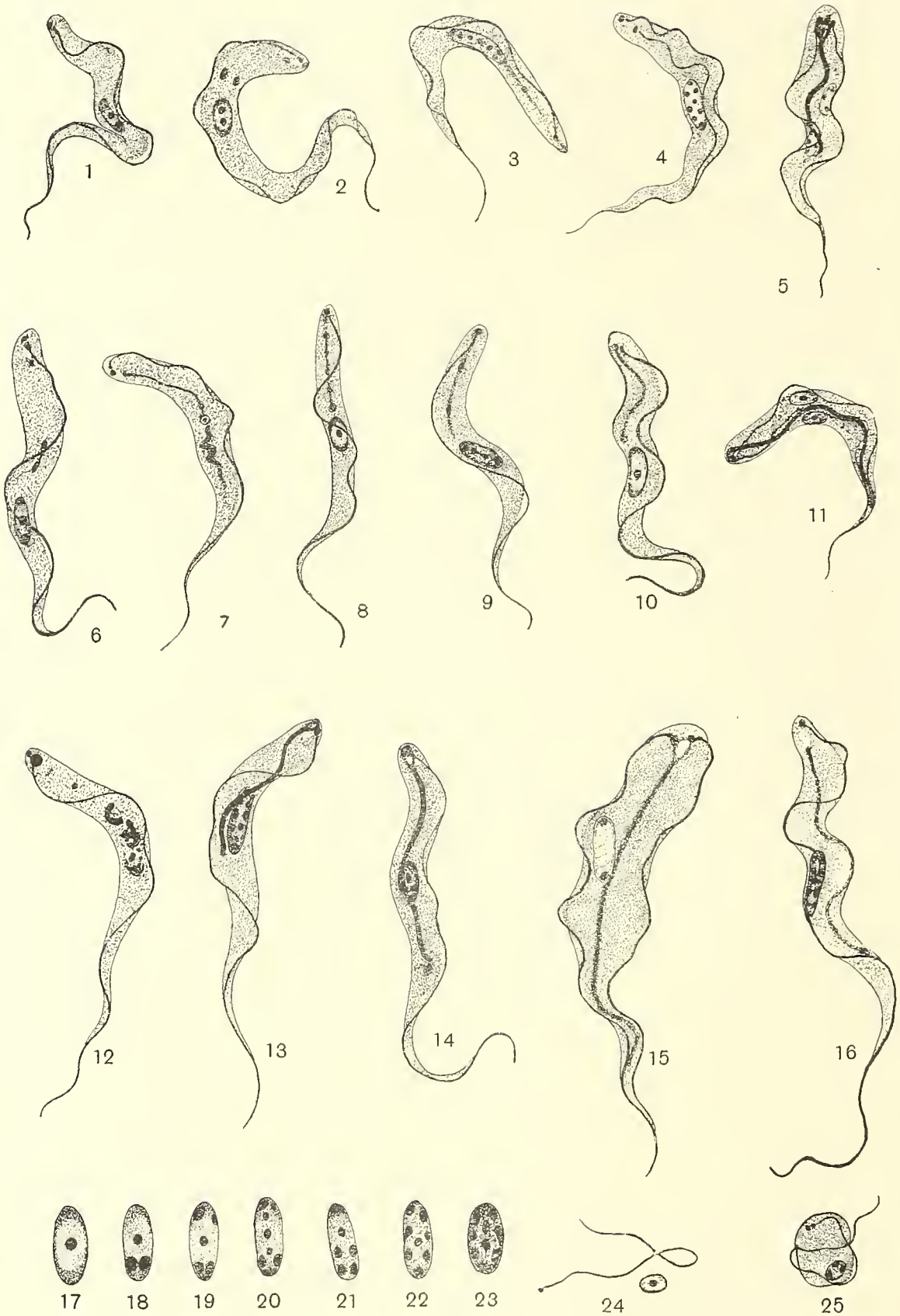
Figs. 17—23. Various tropho-nuclei shewing the increase in the number of granules (×4000).

Fig. 24. Free tropho-nucleus and flagellum, 5 hrs. after treatment. The rest of the parasite has disappeared.

Fig. 25. "Rounded up" form, 4 hrs. after treatment.

Figs. 26—44. Drawn from dry films stained with Giemsa, except when otherwise stated.

Fig. 26. Indifferent type of trypanosome before treatment. (Std. Azur.)



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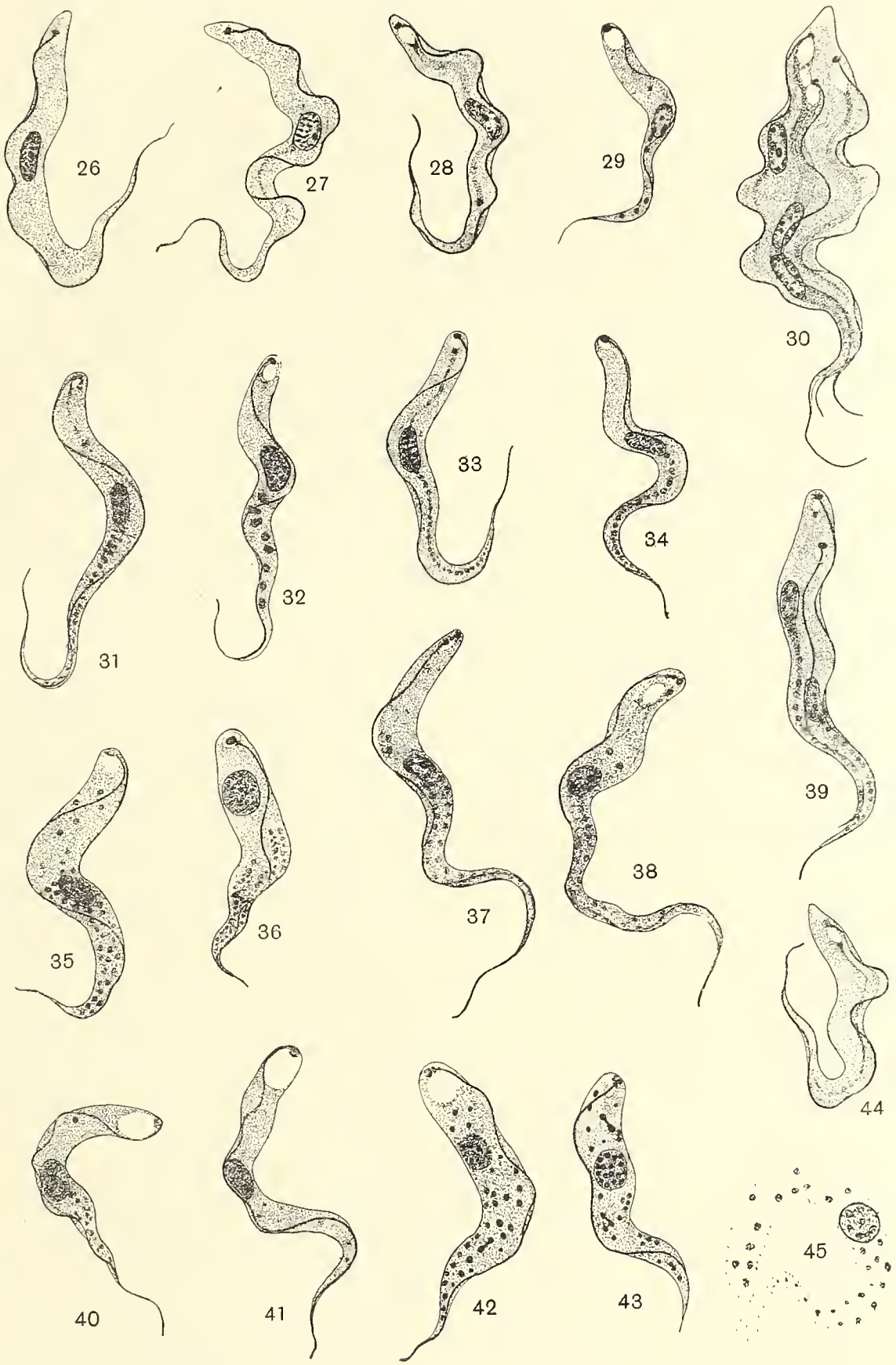
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Fig. 27. Trypanosome shewing trace of axial filament anterior to the tropho-nucleus. (Std. Azur.)

Fig. 28. Parasite shewing well-developed axial filament with both anterior and posterior granules, the latter in relation with the kineto-vacuole.

Fig. 29. Trypanosome, 1 hr. after treatment, shewing enlargement of the kineto-vacuole, and extrusion of granules from the tropho-nucleus.

Fig. 30. Large tri-nnclear form shewing two well-marked axial filaments one of which seems to be dividing.

Fig. 31. Trypanosome shewing various stages in the division of the granules, also dividing kineto-nucleus.

Fig. 32. Trypanosome, 2 hrs. after treatment, shewing the presence of large chromatoid masses in the anterior part of the cell.

Fig. 33. Trypanosome, 2 hrs. after treatment, shewing a row of granules arranged along the degenerating axial filament.

Fig. 34. Drawn from same slide as the preceding figure; shewing the presence of granules in the anterior part of the parasite.

Fig. 35. Stumpy form, 3 hrs. after treatment, shewing extrusion of granules from the tropho-nucleus which are passing forward and crowding in the anterior part of the cell.

Fig. 36. Stumpy form, 3 hrs. after treatment, in which all the granules have crowded to the anterior extremity of the cell.

Fig. 37. Dividing form, 2 hrs. after treatment, in which there is evidence of two axial filaments in the two longitudinal rows of granules in the anterior part of the cell.

Fig. 38. Similar form to the preceding one.

Fig. 39. Dividing form with two tropho-nnclei, each with a well-marked row of granules extending to the anterior extremity.

Figs. 40—45. Trypanosomes 4 hrs. after treatment with arsenophenylglycin.

Fig. 40. Small form shewing enlargement of the kineto-vacuole, and presence of small granules in the cytoplasm.

Fig. 41. Advanced stage of degeneration in which the granules have almost disappeared, being dissolved in the cytoplasm.

Figs. 42—43. Stumpy forms containing nnmerous granules in both the anterior and posterior regions of the cell.

Fig. 44. Trypanosome in which the tropho-nucleus has disintegrated and disappeared. The axial filament is faintly shewn.

Fig. 45. Last stage of degeneration in which the nnclens has become free and the rest of the cell fragmented.

NOTES ON *TRYPANOSOMA LEWISI* AND ITS RELATION TO CERTAIN *ARTHROPODA*.

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and N. H. SWELLENGREBEL, Ph.D.

(*From the Quick Laboratory, Cambridge.*)

(One Text-figure.)

The following notes on the biology of *Trypanosoma lewisi* are the result of experiments extending over the last two years. They are arranged under the following heads:

I. The prevalence of *T. lewisi* in rats under natural conditions.

(*a*) *Endemic foci.* Many observers have reported upon the natural prevalence of *T. lewisi* in rats. The statistics relating to this prevalence of the parasite cannot always be compared, because of their being based on observations made in different ways. Thus Lingard (1895) reported on all the rats he examined irrespective of their age, while Petrie and Avari (1909) only noted the presence of the parasite in young rats.

Working in Bombay Vandyke Carter (1887) found 12 % of the rats infected, Lingard (1895) about 35 %, Petrie and Avari (1909) about 45 %; in the Philippine Islands Musgrave and Clegg (1903) found that about 65 % of the rats at Manila harboured the parasite at certain times of the year; in the United States Swingle (1907) at Nebraska found four out of seven infected, while Yakimoff (1907) in St Petersburg found a higher proportion infected in the summer months than in the winter but he gives no figures.

Prof. Nuttall kindly allows us to report upon the results of the similar observations which he conducted with one of us (C. S.) during 1909 in Cambridge, where 25 % of rats (*M. decumanus*) of all ages were found to be infected.

Having regard to the manner in which all of these percentages of infection were obtained it would appear that the prevalence of *T. lewisi* varies considerably in different parts of the world. The figures given by Vandyke Carter, Lingard, and Petrie and Avari for Bombay indicate moreover that the prevalence of the parasite may vary in a given locality from time to time.

(*b*) *Variation of incidence in endemic foci* (*localised endemicity.*) Carter, Musgrave and Clegg, and Swingle have observed a marked localised endemicity of the infection, and our observations conducted in Cambridge also reveal the local character of rat trypanosomiasis.

Thus, 20 young rats from Comberton, Cambs, shewed no trypanosomes, while a large percentage from Cherryhinton Brook, Cambs, were found to be infected. We at an early date learnt to know the particular localities from which we could rely for a supply of *T. lewisi* infected rats in and about Cambridge.

Of the Cambridge rats which were infected 12 % were young, 33 % adults, and 29 % old animals.

II. The relation of Ectoparasites to the prevalence of *T. lewisi* in rats under natural conditions.

Owing to the fairly constant association in nature of *T. lewisi* and fleas, it has been assumed that fleas are concerned to a considerable extent in the transmission of the trypanosome from rat to rat. The association of *T. lewisi* with flea prevalence in rats has been demonstrated very clearly by Swingle (1907) and Nuttall (1908) and more recently by Petrie and Avari (1909) who report upon the examination of a very large material which was rendered accessible to them through the investigations of the Indian Plague Commission. Petrie and Avari found however that the prevalence of *T. lewisi* was inversely proportional to the flea prevalence, although so generally associated with it.

The prevalence of fleas on trypanosome-infected rats from Cherryhinton Brook, Cambs, was very noticeable. On the other hand we failed to find a single flea on rats from Comberton, Cambs, or on 20 rats imported from Berlin, none of which harboured trypanosomes.

The species of fleas which we have found associated with *M. decumanus* infected with *T. lewisi* are *Ceratophyllus fasciatus*, *Ctenophthalmus agyrtes*, and *Pulex irritans* (one on one occasion in company with *C. agyrtes*). We have also captured on dogs *Ctenocephalus canis* harbouring parasites indistinguishable from *T. lewisi*. This species of flea is one which is also occasionally captured on the rat (Tiraboschi).

Some observers believe *T. lewisi* is conveyed from rat to rat under natural conditions by the rat-louse (*Haematopinus spinulosus* Burm.), but no direct association has been recorded.

Of the other blood-sucking ectoparasites which have been found on rats, none are likely to be more than occasional transmitters of trypanosomiasis because they occur rarely on rats as compared with fleas and lice. The ectoparasites of rats are comprised in the following lists:

Fleas found on *Mus decumanus*, *M. rattus* and *M. musculus*.

(Given by Rothschild 1910, p. 89.)

Dermatophilus penetrans, Linnaeus.
„ *caecata*, Enderlein.
Echidnophaga gallinaceus, Westwood.
„ *myrmecobii*, Rothschild.
„ *murina*, Tiraboschi.
„ *liopus*, Rothschild.

Pulex irritans, Linnaeus.
Xenopsylla cheopis, Rothschild.
„ *brasiliensis*, Baker.
Hoplopsyllus anomalus, Baker.
Ctenocephalus canis, Dugès.
„ *felis*, Bouché.
Ceratophyllus fasciatus, Bosc.
„ *londiniensis*, Rothschild (=*italicus*, Tiraboschi).
„ *anisus*, Rothschild.
„ *penicilliger*, Grube.
„ *niger*, Fox.
Pygiopsylla hilli, Rothschild } perhaps not found on *M. rattus* but on
„ *rainbowi*, Rothschild } *M. assimilis* only.
Chiastopsylla rossi, Waterst.
Neopsylla bidentatiformis, Wagner.
Ctenophthalmus agyrtes, Heller.
„ *assimilis*, Taschenberg.
Ctenopsylla musculi, Kolenati.
Hystrichopsylla tripectinata, Tiraboschi.

Lice and *Acarines* occurring on *M. decumanus* and *M. rattus*.

(Given by Tiraboschi 1903—4, p. 161, and by Shipley 1909, p. 68.)

Lice.

Hoplopleura acanthopus, Drury.
Haematopinus spinulosus, Burmeister.
Pediculus capitis, Nitzsch.
Haematopinus precisus, Neumann.

Ticks.

Ixodes ricinus, Linnaeus (larvae reported by Nuttall in England: noted by Neumann).
Hyalomma aegyptium, Linnaeus (larvae from India reported by Nuttall).
Rhipicephalus sanguineus, Latreille (adults identified by Nuttall; see Shipley).

Demodicidae.

Demodex musculi, Oudemans (other varieties have been found on rats (Hahn), and we find one species very common in Cambridge).

Gamasidae.

Laelaps agilis, Koch.
„ *echidninus*, Berlese.
„ *stabularis*, Koch.
Myonyssus decumani, Tiraboschi.
Notoedres alepis, Railliet-Lucat.

III. Experiments demonstrating the part played by Arthropods in the transmission of *T. lewisi* from rat to rat.

We have experimented with fleas (*Ceratophyllus fasciatus* and *Ctenophthalmus agyrtes*), lice (*Haematopinus spinulosus*), bugs (*Acanthia lectularia*), ticks (*Ornithodoros moubata*, nymphs and adults), and a Gamasid mite (*Gamasus* sp.). Only in the case of fleas did any transmission of *T. lewisi* take place by the act of transferring the arthropods from an infected to a non-infected rat. The result of these experiments are recorded in the present paper.

(i) *Experiments with fleas.*

(*a*) *By previous authors.* Rabinowitsch and Kempner (1899) and Nuttall (1908) were the first to regard fleas as transmitting agents of *T. lewisi* and to demonstrate that they convey the infection, and their experiments have been confirmed by Minchin and Thomson (1910).

Minchin and Thomson have recently proved that fleas if fed on an infected rat do not infect susceptible animals until about a fortnight afterwards. This is conveniently described as 'distant transmission' or 'late infectivity of the fleas.' The fleas then continue able to infect for a long time, possibly till the end of their life. From this it is obvious that fleas caught haphazard in nature may have already passed the latent non-infective period and may exhibit the power of distant continuous transmission.

(*b*) *Our experiments.* In all of our experiments the arthropods were allowed to feed on an animal infected with *T. lewisi*, after which they were transferred to a clean animal, a variable interval of time being allowed to elapse before they were transferred from the infected to the clean animal.

We performed 138 experiments with fleas and 22 of these (about 16 %) gave a positive result.

On adding up the numbers of the fleas which were used in the attempts to transmit infection, we find that 324 were used when positive results were obtained, while 1270 were used with negative results. That is, about 80 % were non-infective. Of the remaining 20 % concerned with the positive results it is safe to assume that some at least were also non-infective.

We had positive results in 20 experiments and as one flea at least in each experiment must have been infective, at least 20 must have been infective, *i.e.* about 2 % of the total number used.

Therefore at least 2 % and not more than 20 % of our fleas were infective.

We would like here to add the results of flea infection experiments which one of us (C. S.) obtained before the publication of Minchin and Thomson had appeared, since they concerned cases of what may be regarded as 'distant transmission' observed in January, 1909.

Experiment 1. About 20 fleas of two species (*C. fasciatus* and *C. aegyrtes*) from an infected wild rat were placed upon a tame white Rat XV. Four days later Rat XV died and about six fleas from it were then placed on a 'clean' Rat XI. Two days later this rat died, and the fleas from it were placed on Rat XVII which became infected in six to ten days. The fleas were therefore infective six days after taking them from the infected rat.

Experiment 2. 17 fleas from an infected wild rat were put on a 'clean' rat for 12 days when nine were recovered and put on a second 'clean' rat. This became infected in eight days. These fleas were therefore infective 12 days after coming from the infected rat. The first 'clean' rat did not become infected.

The result of the following experiment can be interpreted in the way indicated in the second paragraph of this section (distant continuous transmission).

Experiment 3. 15 fleas taken from an infected rat were placed immediately on a 'clean' white Rat XX. In three days four were recovered and put on Rat *a*; similarly after two and one days the fleas were placed in succession on Rats *b* and *c*. All four rats became infected.

The following experiments also prove distant transmission.

Experiment 4. Rat 5 was placed in a box containing fleas which 14 days previously had fed on an infected rat. Rat 5 became infected.

Experiment 5. Rat *d* was used to feed fleas upon 12 days after they had fed on an infected rat. Rat *d* became infected.

Experiment 6. Fleas from an infected rat were placed on a 'clean' Rat *e* seven days after they had fed upon the infected rat. Rat *e* became infected.

Experiment 7. Fleas from an infected rat A were placed on a 'clean' rat B 17 days after they had fed on the infected animal A. Rat B became infected.

Experiment 8. A rat D††† was placed in a flea box from which an infected rat had been taken three weeks previously. Rat D††† became infected in seven days.

Experiment 9. Fleas were fed on an infected animal for an hour and were then fed daily on a series of 'clean' animals from the 4th—33rd day, but none of them became infected. On the 34th day the fleas were put on Rat C which became infected.

It seems therefore characteristic of the flea transmission of *T. lewisi* that it occurs after the lapse of a period during which the fleas are incapable of communicating the parasite. This result is in accord with that obtained by Minchin and Thomson.

We are however inclined to think that infection *occasionally* follows the immediate transference of the fleas to a 'clean' animal, in other words that the so-called mechanical transmission occurs and that it may be followed by the latent or non-infective period which precedes the infective period when distant transmission takes place. In support of this view we would cite the following experiment.

Experiment 10. Fleas were used which had been raised in the laboratory, in the manner advised by the Indian Plague Commission, in large glass boxes in which a flea-infested rat had been placed. Under such conditions the fleas readily multiply in the filth which soon collects in the box. 11 of such fleas were fed for one hour on a rat whose blood had shewed *T. lewisi* for 12 weeks. After 24 and 72 hours the fleas were again fed on a 'clean' Rat D. Rat D became infected in seven days. After this the fleas were fed at intervals on a series of 'clean' animals, but they did not infect the rats again until the 34th day.

In all experiments where we excluded the possibility of 'mechanical transmission' five out of nine rats became infected, but where the possibility was not excluded five out of six experiments had a positive result. The difference in the results observed may have been due to mechanical transmission having been the means of infecting in some of the experiments. The average number of fleas for each experiment was the same in the two series.

If mechanical transmission of *T. lewisi* by fleas takes place we thought that *T. brucei* should also be transmitted mechanically, but the results of six experiments were negative. We allowed fleas to feed on a rat heavily infected with *T. brucei* and then transferred them to clean rats, but in no case did these rats become infected.

We obtained no evidence of hereditary transmission of trypanosomes in fleas although we experimented with a large number (about 60) raised from the larva removed from a flea-breeding box in which an infected rat had been kept for many weeks. The flea imagines produced no infection when placed on 'clean' rats.

Certain conditions may influence the transmission of *T. lewisi* by fleas. For instance Petrie and Avari say that the hot season is the more favourable to transmission because they found fewer fleas *on* the rats and more cases of infection. We think that a favouring factor is connected with the 'wildness' of the flea, for we found that with fleas

taken from infected wild rats transmission was obtained much more readily than with those bred and fed in the laboratory. Sir David Bruce and his collaborators (1910) have noted a similar phenomenon in the transmission of *T. gambiense* by *Glossinae.*

Fleas seem to be able to transmit infection whether they have fed on a rat recently infected or on one which has been long infected. We have obtained transmission of *T. lewisi* from a rat which had only been infected one to four days, and from a rat on the other hand which had been infected for three and a half months.

(*c*) *The mechanism of infection.* It has been assumed that fleas infect rats with *T. lewisi* during the act of blood sucking, the parasites being introduced into the host through the fleas' mouth parts. In view of this assumption it seemed somewhat strange that we should only find parasites in the fleas' hindguts: as has been described in a previous paper by us. If we assume that after all the 'small trypanosomes' are the infective agents we could hardly expect them to be carried forward from the hindgut through the pyloric valve and the proventriculus to the mouth parts, for both valves are designed to prevent regurgitation (*see* Jordan and Rothschild, 1908). Moreover we have never found small trypanosomes in the midgut of fleas whose hindgut has been swarming with this form of the parasite. As we have seen we have succeeded repeatedly in infecting rats upon which infective fleas were placed and allowed to feed naturally, that is by being allowed to crawl about and remain upon the host. With the object of learning more about the mechanism of infection attempts were repeatedly made by us to infect rats by allowing infective fleas to bite them through fine gauze, but the results were always negative: thus

Experiments 11—16. A large number of infected fleas (20—30) in six separate experiments were first starved for several days and were then fed respectively on six clean rats through fine gauge. Most of them were observed to feed and could be seen distended with blood afterwards, but none of the 'clean' rats became infected; but when the same fleas were afterwards placed upon another lot of six clean rats and allowed to feed naturally, the latter all became infected.

Experiment 17. Eighteen fleas which had fed on an infected rat E for one hour were fed through fine gauze on a series of clean rats for about a month without any result, but when the only two which survived this time were *put on and left on* a clean rat and allowed to feed naturally, this latter became infected.

Experiment 18. A batch of about 200 fleas were first starved for a few days and were then fed on eight clean rats through fine gauze. After this they were let loose on six other clean rats. Whereas none of the rats on which the fleas fed through gauze became infected, the rats on which they were let loose all became infected.

We are therefore doubtful about the act of biting conveying 'late' infection.

As it seemed to us that late infection was not conveyed by the bite of the flea, we thought that it might ensue from the act of the rat biting and swallowing the flea. We therefore performed the following experiment.

Experiment 19. A 'clean' rat D XII was fed with bread in which was incorporated about 12 fleas which were thought to be infective, as 96% of their fellows from the same flea-box exhibited small trypanosomes in their hindguts. However no infection resulted.

It is certainly possible that the mechanism of late infection is a *contaminative* one, due to the voiding of the infective agents on to the skin of the rat. It has been stated that during the act of feeding certain fleas squirt excrement from their recta, but we can say that this is not so in the case of *C. fasciatus* or *C. agyrtes* for we have observed many hundreds in the act of feeding and it has never then occurred. Moreover we have never observed that the coat of a rat infested with fleas becomes soiled with their excrement, although we would note here that Brumpt has found the coats of heavily infested mice to be badly soiled by flea excrement. Again, it is very difficult to find any trypanosome forms in the voided excreta of fleas which are even heavily infected with the small trypanosomes in the hindgut and rectum. If infection were due to contamination, one would expect rather a large number of the parasites to be excreted, especially if there were many in the rectum. On the other hand the fact of the presence of the parasites in the hindguts and recta of fleas seems in itself to lend colour to the idea of the infection of the rat being due to contamination. We would also mention here that Manteufel (1909) had no difficulty in infecting rats by placing blood infected with *T. lewisi* on their skins, while Hindle (1910)[1] has lately obtained infection of rats in a similar way by *T. gambiense*. We cannot say what the mechanism of infection really is, but our experiments would appear to indicate that the bite of the flea inoculating the parasite into the rat is not the usual mode of transference.

On the other hand the following experiment seems to indicate that 'mechanical' transmission is effected by the bite.

Experiment 18. Ten fleas from an infected rat were fed within 24 hours on a clean rat *through gauze*, with resultant infection. After this the fleas were

[1] In press, to appear shortly in the *Brit. Med. Journ.*

similarly fed on a series of animals with negative results for about four weeks. They were then put on another rat and left on it and this became infected.

This mechanical transmission by the bite is probably due to the inoculation of the blood forms from the mouth parts of the flea, but there must be even here a close adaptation of the parasites to their environment, for this mechanism is ineffective to transmit *T. brucei* under similar conditions.

Our experiments seem to indicate that the mechanism of infection is not the bite of the flea for 'late' transmission, but that it is so for 'mechanical' transmission.

(*d*) *The relation between the developmental cycle of T. lewisi in the flea and infection by the flea.* It stands to reason that the bodies of potentially infective fleas must contain *T. lewisi* in some form or other, and consequently that the flagellates should be found in the insects upon microscopic examination. It should also be possible to find some forms of the trypanosomes in fleas during the latent period prior to their becoming infective in cases where distant transmission takes place. We have examined a large number of fleas with the object of discovering potentially infective parasites which they might contain, with the following results:

I. Having removed 75 fleas (*C. fasciatus*) from breeding-box 'A' in which an infected rat had been kept for some weeks, they were fed on a series of 'clean' rats during a period of 14 days. None of the clean rats became infected through the agency of the fleas. All of these fleas were dissected and examined during a period lasting for 14 days after they had been removed from the vicinity of the infected rat. In only two of the 75 fleas were flagellates encountered. These two fleas had not been in contact with the infected rat for seven and nine days. The flagellates were small in numbers, occurred in the hindgut, and were of the type described on p. 374.

The examination of the fleas' salivary glands, ovaries, fat-bodies, Malpighian tubes and coelomic fluid gave negative results. The object of first feeding the fleas on 'clean' rats was of course to get some clue as to the presence of infective parasites, or by discovering the mechanism of infection to deduce the whereabouts of the parasite in the insect.

II. Another batch of fleas to the number of 239 were removed from breeding-box 'B' in which an infected rat had been confined for about four weeks. The fleas were divided into two lots (*a*) and (*b*). The fleas in lot (*a*), 20 in number, were dissected and hypothetical infective forms were looked for in all of their organs with the result

that 96 % of the insects were found to contain large numbers of 'small trypanosomes' in the hind gut.

The fleas in lot (*b*), 219 in number, were used for infection experiments on rats. They were placed on 14 clean animals about four days after taking them from the vicinity of the rat infected with *T. lewisi*. About 16 were placed on each rat. Eight of the 14 rats became infected; 45 % of the fleas distributed on six rats not being infective, *i.e.* although 96 % of the fleas in lot (*a*) contained 'small trypanosomes' only 55 % (at the most) of the fleas in lot (*b*) proved infective. We do not know upon what this discrepancy depends.

Since we found no other forms than the small trypanosomes in the hindgut we assumed that these might represent the potentially infective forms, but this assumption gained no support from experiments in which two rats respectively were inoculated subcutaneously and intraperitoneally with 'small trypanosomes' derived from the hindguts of four fleas and yet did not become infected.

We therefore thought that possibly the parasites detected had not reached a stage when they were infective under the conditions in which the experiments were carried out. Although we found no parasites in the infective fleas other than those encountered in the hindgut, an emulsion of the salivary glands and alimentary tracts of 11 fleas from an infected flea-box were respectively inoculated into two rats, but the results were again negative.

We have already said that we think the 'small trypanosomes' in the hindgut of the flea are the final forms which *T. lewisi* takes in the flea, and that it is they which find their way back to the rat when the flea has passed the latent period of non-infectivity and become infective, (late infection).

The forms of the parasite which are found in the flea prior to the 'small trypanosomes' correspond in the time of their occurrence to the time when the fleas are non-infective.

We therefore believe that while the 'small trypanosomes' are inherently able to grow in the rats' blood and infect the rat, the forms which precede them are not capable of doing so. This is we think analogous to the relation of the malarial parasite to its power of infection at different stages of its life cycle, and not to a bacterial culture, which would be infective continuously.

We agree with Novy and MacNeal in the case of *T. lewisi* that the growth in the insect is similar to the growth on agar in 'artificial culture,' and therefore that their conception of the insect being a flying

culture tube is to a certain extent justified. The differences which exist are differences of degree, not of kind: the development of *T. lewisi* in the flea proceeds a further stage than it does on agar.

We quote here an observation which may have some significance to the biology of trypanosomes. We found in an experiment that several fleas taken from a heavily infected rat and then fed for a fortnight on a rat which was immune to *T. lewisi* were nevertheless infective; which shews that serum which would have prevented the growth of *T. lewisi* in the rat has no power to do so in the flea.

During the initial period of infectivity of the flea, during the first day when *mechanical* transmission is effected, the trypanosomes are found in the midgut of the flea, and may possibly be the forms which cause infection, perhaps by regurgitation of the gut contents, but we think that probably this mechanical transmission is effected by a 'wet' proboscis, or in some cases by the rat chewing up a flea which is full of *T. lewisi* and becoming infected by way of the mucous membrane of the alimentary tract.

(ii) *Experiments with lice.*

(*a*) *By previous authors.* Many workers have apparently succeeded in transmitting *T. lewisi* by the agency of lice; namely, MacNeal (1904), Swingle (1907), Nuttall (1908), Manteufel (1908), Baldrey (1909), Bensen (mentioned by Baldrey 1909), and Breinl and Hindle (1909).

On the other hand failures to transmit the infection by lice have been common. Thus, MacNeal records but one out of a number of experiments which he performed which was successful. Swingle also succeeded in infecting rats by means of lice, but he does not state how many times he failed to do so. Manteufel (1908) although he seems several times to have been able to transmit *T. lewisi* by lice, yet records 40 failures. Prof. Nuttall (1908) succeeded once (with 60 lice) and failed once (with 40 lice). Baldrey (1909) has succeeded and failed, but gives no figures. Breinl and Hindle (1909) quote three positive experiments but they also had negative results.

Rabinowitsch and Kempner (1899) and Prowazek (1905) could not succeed at all in their efforts at transmission of the parasite.

Further observations are required, but those which we review certainly indicate that transmission experiments by means of lice frequently yield negative results, even when large numbers of lice are transferred from infected to uninfected animals.

(*b*) *Our experiments.* We ourselves have performed 17 experiments all of which gave a negative result, although we modified the conditions of the experiments in various ways.

With regard to these experiments with lice, they have for the most part been conducted with considerable numbers of insects, and we believe that it is only by large numbers that infection is produced. In our 17 negative experiments an average of 67 lice were used in each experiment. It therefore seems more unlikely than ever that lice play any important part in nature in the dissemination of the disease.

It is conceivable that 'distant transmission' may occur in the case of lice in the manner observed in the case of fleas, but there is no trustworthy evidence to prove it. Whilst Baldrey (1909) records one experiment in which he says both mechanical and distant transmission was proved, the experiment has little value since he says that many of his experimental rats were spontaneously infected. Although we repeated Baldrey's experiment, we failed to obtain a positive result. We only carried out one experiment as follows:

Experiment 21. One week after the trypanosomes had disappeared from microscopic observation in the blood of a white rat several lice (about 30) were removed from the rat and after 24 hours, were placed on a 'clean' white rat, but no infection followed, that is, no distant transmission occurred. We will here remark that it was in lice out of this batch that we found the developmental forms of *T. lewisi* described on pp. 378—384 (This *Journal*).

More experiments are needed on this subject. We think that the adoption of Manteufel's or Breinl and Hindle's technique may favour the obtaining of positive results. Manteufel prevented the lice which he used from being eaten by the rat on which they were put by immobilising the rat. Breinl and Hindle transferred the lice which they used to lousy uninfected rats, by which means the transferred lice did not attract the attention of the rat and so get destroyed before they had had time to transmit the parasite to the rat.

(*c*) *The relation between the developmental cycle of T. lewisi in the louse and infection by the louse.* The connection between infection and the morphological character of the ingested trypanosome in lice may ultimately be found to be analogous to that in the flea. The morphological characters of the parasite in the louse have been very variously described by different authors, but a cycle of development terminating with a crithidial or herpetomonad stage seems to have been seen by most observers. We ourselves have recently (1910) described the behaviour of *T. lewisi* in the louse as being exactly

similar to that in culture, which agrees with most other workers' observations. We noted that either owing to lice being very short-lived, or to the parasite dying out in lice relatively rapidly, or to some other cause, the growth was found to persist for only a very short time in the louse.

With regard to the coelomic crithidial forms in the louse which have been described by Baldrey, it is possible that they were independent flagellates, and we think that the developmental cycle which the same author describes is partly described from young blood forms of *T. lewisi* ingested by the louse. Baldrey says indeed that the developmental forms are only to be found in lice which have fed on rats just infected with *T. lewisi.*

We would like to emphasize here that the development of the trypanosome in the louse is a very rare occurrence. One of us has previously reported (C. S. 1910) not finding any stage of development whatever in 275 lice, while the other has examined a great number in Holland with negative results. Other observers also have never observed any cycle of development. Thus, Breinl and Hindle say that they examined so many lice from rats infected with *T. lewisi* with negative results that they were about to give up the search when they suddenly came upon developmental forms. Notwithstanding this these authors go on to make the remark that it seemed surprising 'that Nuttall and his co-workers had failed' in the search.

Whatever the cycle of development of *T. lewisi* in the louse may be, it is not very clear as to its connection with infection of rats by lice, as will be seen in the following list of observations made by different authors. Prowazek (1905) and Rodenwaldt (1909) both observed a developmental cycle in lice, but could not succeed in transmitting the parasite by their agency. We have also, as stated above (1910), found a developmental cycle in the louse, but were unable to transmit the parasite with such lice.

Baldrey (1909) however found the developmental forms in lice, and succeeded in transmitting *T. lewisi* by means of them. He correlated what he thought was 'early' and 'late' transmission with different stages in the developmental cycle of the parasite. Similarly Breinl and Hindle (1909) succeeded in transmitting *T. lewisi* from rat to rat by lice and they saw developmental stages of *T. lewisi* in such lice.

On the other hand some of those observers who could find no sign of any development of *T. lewisi* in the louse, were able to transmit infection from infected to non-infected rats by the louse, while others

could not. Those who succeeded were MacNeal (1904), Nuttall (1908), Manteufel (1908), and Breinl and Hindle (1909) who at that time could not find the developmental changes. Those who failed were ourselves who at that period could not find the developmental stages, although we have since succeeded in doing so.

(iii) *Experiments with other arthropods.*

We carried out two experiments with *Gamasus* sp., a mite which is occasionally found on rats; two with bugs (*Acanthia lectularia*) and one each with *Ornithodoros moubata* nymphs and adults; but in all cases with negative results. It is of course conceivable that if more of these experiments had been performed a positive result might have been attained.

In all of these experiments the arthropod was taken from a well-infected rat and then placed on a 'clean' rat. The bugs which we used, on being dissected, shewed the developmental forms which we have described on p. 386, while the tick-nymphs were used seven days after they had come from an infected rat and their guts were full of active trypanosomes.

Experiment 22. Four *Gamasus* which were taken from a wild rat infected with *T. lewisi* were immediately placed on a 'clean' white rat, but no infection resulted.

Experiment 23. Two *Gamasus* were taken from a rat heavily infected with *T. lewisi* and put on a 'clean' white rat, but no infection followed.

Experiment 24. About four days after some bugs (*Acanthia lectularia*) had fed on a rat infected with *T. lewisi*, they were allowed to feed on a 'clean' white rat. The result was negative.

Experiment 25. About six days after some bugs had fed on an infected rat, they were fed on a 'clean' white rat, with negative results.

Experiment 26. Several nymphs of *Ornithodoros moubata* were fed on an infected rat and seven days after were fed on a 'clean' animal, but no infection resulted.

Experiment 27. Two adult *O. moubata* which had recently fed on a rat infected with *T. lewisi* were fed on a 'clean' white rat which however did not develop infection.

We thus see that, although we have previously (1910) shewn that *T. lewisi* persists for a definite period in the gut of the bug and the adult tick *O. moubata*, yet we could not obtain infection with these arthropods. It is certainly possible that these arthropods may occasionally transmit the infection, and that this depends on the behaviour

of the parasite in their bodies after being ingested, but we have not yet been able to obtain infection with arthropods in which subsequent dissection has shewn the persistence of *T. lewisi*.

IV. Description of a peculiar form of *T. lewisi*.

We would like to make brief mention here of a curious form of trypanosome found in the rat's blood by one of us. It is characterised by an enormous elongation of the posterior end of the body, the total length of the protozoon being 45 μ. The flagellum is short, and stains deeply red with Giemsa's stain, and though it does not end in the blepharoplast which is very large and spherical, yet no basal granule is evident at its proximal end. The nucleus is situated in the anterior part of the cell

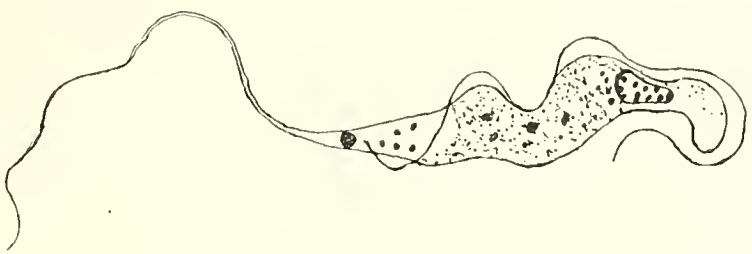

Fig. 1. ×2645.

and shews the achromatic substance perfectly with disseminated chromatic granules in it. The protoplasm possesses a distinctive band of dense cytoplasm in its middle third, and also a riband of dense protoplasm at the anterior end along the flagellar side. Posterior to the nucleus is a very distinct band of very loose spongioplasm. Disseminated all through the protoplasm are a number of prominent chromidia.

Wendelstadt and Fellmer have recently found such forms in rats inoculated with *T. lewisi* which have been passed through reptiles and other cold-blooded animals. Its presence in a spontaneously infected rat is therefore suggestive. Thomson (cited by Woodcock) has also previously seen these forms, and other organisms with a considerable prolongation, but not so filiform, have been encountered by one of us (N. H. S.).

Summary and Conclusions.

1. *Trypanosoma lewisi* was found in 25 % of rats caught in and about Cambridge. Of these rats 12 % were young, 33 % adult, and 29 % old. In this region the endemicity was localised to certain areas.

2. Fleas (*C. fasciatus* and *C. agyrtes*) were constantly found associated with wild rats infected with *T. lewisi*. We think that these two species of fleas are the chief carriers of the parasite from rat to rat in this region.

3. We tried transmission experiments with fleas (*C. fasciatus* and *C. agyrtes*), lice (*H. spinulosus*), bugs (*A. lectularia*), ticks (*O. moubata*, nymphs and adults), and *Gamasus* sp., but only obtained a positive result by the agency of fleas.

4. In the transmission experiments the arthropod was transferred from an infected to an uninfected animal. 16 % of 138 experiments with fleas were successful, 80—98 % of the fleas used were non-infective, while 2—20 % were infective.

5. Distant transmission occurs with fleas. This was observed by one of us in January, 1909.

6. 'Mechanical' transmission occurs sometimes with fleas.

7. Infective fleas do not transmit the power of infection to their young.

8. The mechanism of infection in the case of mechanical transmission is by the bite of the flea; in the case of the distant infection it is not so.

9. The infective forms of *T. lewisi* for distant transmission are probably the 'small trypanosomes' which we have described elsewhere (1910). The forms of *T. lewisi* found prior to the small trypanosomes in the developmental cycle are not infective.

10. The development of the trypanosomes in the flea is not interfered with by the flea feeding on a rat immune to the disease.

11. Seventeen experiments with lice were performed, but no transmission was obtained by their agency. 1139 lice were used. No confirmation of Baldrey's infective cycle in lice could be obtained.

12. We could trace no connection between transmission of infection by lice and the presence in them of 'developmental forms.'

13. No transmission of *T. lewisi* by other arthropods was obtained. These negative results had no connection with the presence in the arthropods of 'developmental forms.'

In conclusion we would like to thank Professor Nuttall for his kindness in inoculating our animals and providing us with the material for our experiments, and for his interest and advice in our work.

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A BIOMETRIC STUDY OF *TRYPANOSOMA GAMBIENSE.*

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(*From the Quick Laboratory, Cambridge.*)

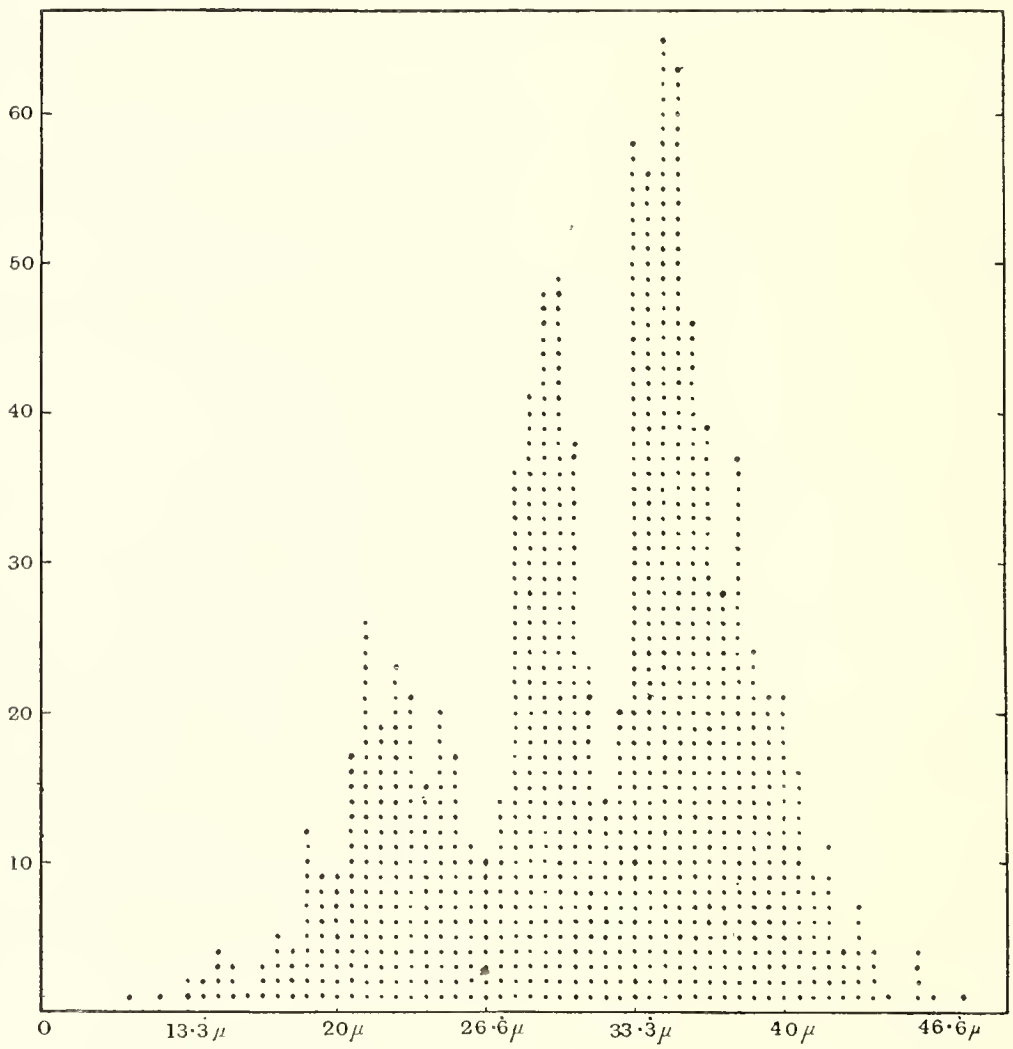
The three forms of *Trypanosoma gambiense* have been described so often that a few words of apology are needed before bringing forward the following note on the subject.

In their memoir on the morphology of this parasite, Salvin-Moore and Breinl (1907) deny the existence of any true dimorphism or trimorphism in *T. gambiense*, but regard the forms, often described as distinct, as merely "arbitrarily chosen examples in a continuous series of dimensions." Although the three types, as shewn by Minchin (1908), appear quite distinct when compared side by side, yet the existence of transition stages between the two extremes of "long" and "stumpy" forms makes it possible that the two latter are merely extreme variations of one type. If this were the case, however, a curve plotted from the dimensions of a reasonably large number of individuals should present only one apex, which graphically expresses the fact that the variation is around one mean point.

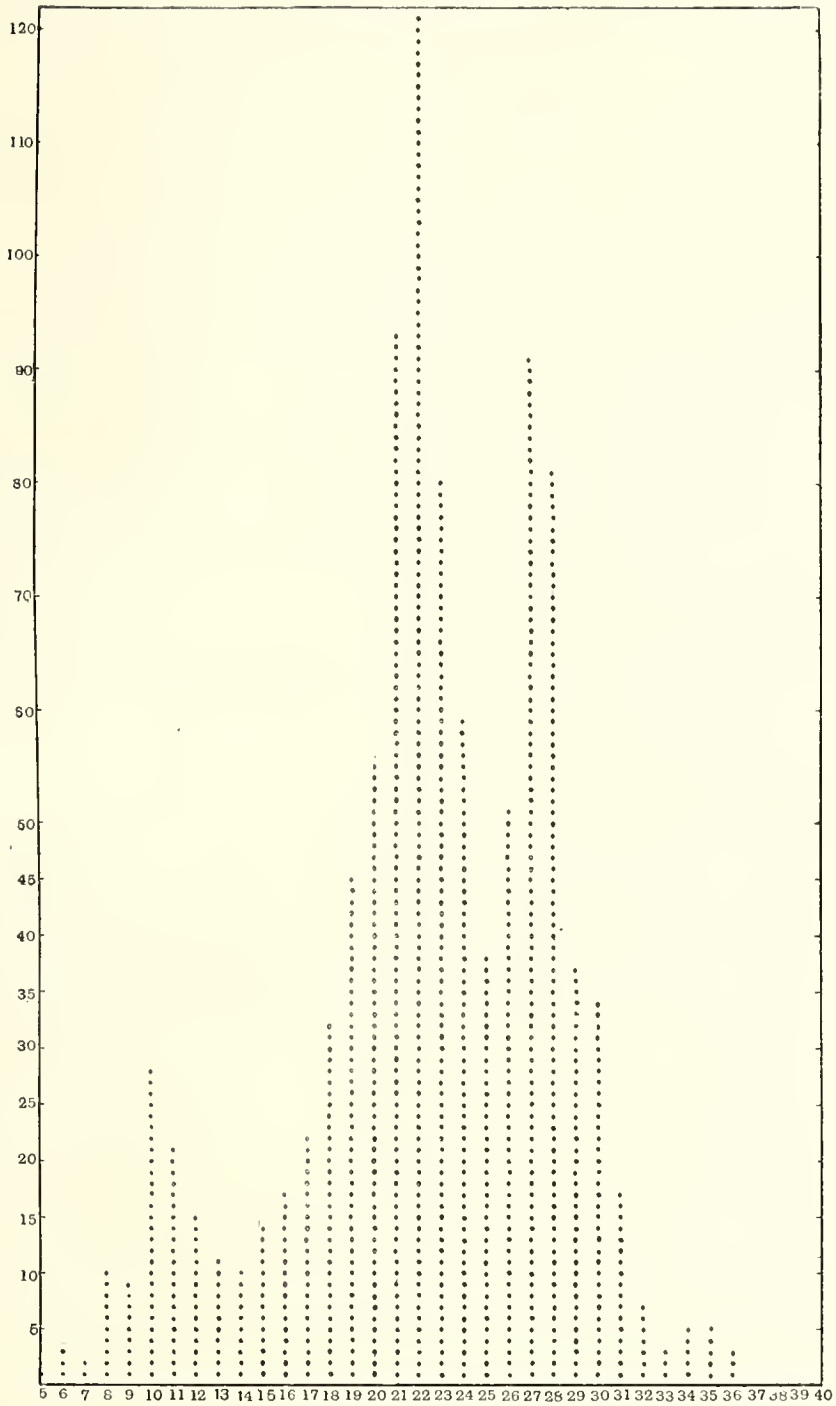
In order, therefore, to determine whether the variations in the size of *T. gambiense* are around one mean point or more, the dimensions of a thousand individuals have been plotted in the form of two curves The method of obtaining these dimensions was as follows:

A film was prepared from the blood of a heavily infected rat containing numerous parasites in each microscopic field. The film was dried, fixed in absolute alcohol, and stained with Giemsa in the usual way. The general outline of the body obtained with this dry film method is far superior to that obtained after employing wet

methods of fixation. A thousand trypanosomes in the middle area of the film were then carefully drawn, with the aid of a camera lucida, to a magnification of 3000 diameters (Zeiss 2 mm. ap., oc. 18), care being taken to avoid missing any of the parasites or going over any part of the slide more than once. When the requisite number of drawings had been made, the length and breadth of each were carefully measured and the dimensions arranged in the form of a table. The length was taken along the axis of the body, from the posterior extremity to the extreme tip of the flagellum, and in order to obtain this measurement a "map measurer" was employed, with the aid of which it is easy to



Curve *A*. Illustrating the relative frequency of trypanosomes of equal length. The length of the parasites is measured along the horizontal line and the number of individuals along the vertical, each dot corresponding to one trypanosome.



Curve *B*. Illustrating the relative frequency of trypanosomes of equal bodily proportions. The ratio $\frac{\text{length}}{\text{breadth}}$ is measured horizontally, and the number of individuals vertically, each dot corresponding to one trypanosome.

follow the numerous curves of the trypanosome. The breadth was always measured through the tropho-nucleus, and transversely to the axis.

From the figures obtained as the result of this rather laborious series of measurements, two curves have been plotted, one of which (curve *A*), expresses the relative frequency of trypanosomes of any particular length, and the other (curve *B*), of any particular shape.

In curve *A* the length of the trypanosomes is plotted along the horizontal line, and the number of individuals of any particular length is given by the vertical heights, and it is evident that there are three distinct apices to the curve.

In curve *B* the relation $\frac{\text{length}}{\text{breadth}}$, which expresses the shape of the trypanosomes, is plotted horizontally, and the number of individuals, vertically. Here again there are three distinct apices, although the one corresponding to the "stumpy forms" is much lower than the other two.

These two curves, therefore, each exhibiting three well-marked apices, conclusively prove the existence of three distinct types in *Trypanosoma gambiense*.

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NORMAL AND ABNORMAL MORPHOLOGY OF *TRYPANOSOMA LEWISI*, IN THE BLOOD OF THE RAT.

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(With 15 Diagrams.)

CONTENTS.

When comparing the results of the recent morphological study of *T. lewisi*, we note a general tendency to bring these observations into accordance with the views expressed in Schaudinn's well-known publication (1904). In this paper I propose to discuss the morphology of this interesting flagellate under normal and morbid conditions, and to consider whether the present tendency to adopt Schaudinn's views is justified. Since I shall take occasion to compare my observations with those of other authors, it will not be necessary to give a historical review of the subject. A full list of references will be found in the papers of Rabinowitsch and Kempner (1899), Wasielewski and Senn (1900), Laveran and Mesnil (1901), and Prowazek (1905). I have discussed matters of technique in a previous paper (1910) to which the reader may refer.

1. *Forms of the chronic infection.*

As is well known these forms appear 8—13 days after infection, they no longer divide. It is not necessary to insist on the general aspect, this being well-known from the description of the authors already

mentioned. In the first period of chronic infection curious forms may be seen, with a very elongated hind end (Diagram VI, fig. 2). Prowazek mentions them and considers that they have no supporting fibrillae and are consequently very metabolic.

(*a*) *The protoplasm.* Between the nucleus and the blepharoplast a filament may frequently be observed, staining blue with Giemsa. I have already referred to this structure in an earlier paper (1910) (Diagram I, fig. 1). This filament is probably to be homologised with similar structures found in *T. gambiense* and *T. brucei* (Robertson, 1906). Moore, Breinl and Hindle (1908) do not mention it as occurring in *T. lewisi,* but I have observed it so frequently and under so many conditions of fixation and staining that a mistake can be excluded. Generally the filament is quite continuous, but sometimes it breaks up in a row of irregular granules, which are, however, different from the chromidia, which stain red with Giemsa (Diagram I, figs. 1, 2).

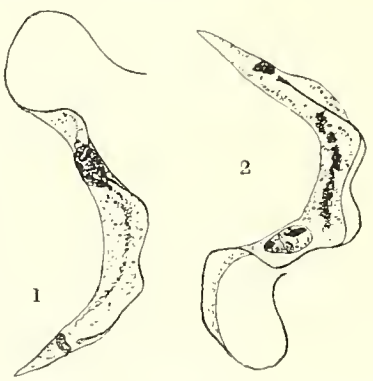

Diagram I.

Fig. 1. Continuous axial filament.
Fig. 2. Axial filament broken up.

The structure of the protoplasm was considered in my previous paper.

(*b*) *The blepharoplast* is a rod-shaped organellum, and can only be studied in preparations carefully stained with Giemsa. If it is over-stained the achromatic part cannot be distinguished from the chromatic part. In many cases the blepharoplast is composed of two chromatic granules, united by a substance staining pink with Giemsa (achromatic substance) (Diagram I). The presence of these two granules does not indicate a division of the blepharoplast, it is a premature division of the centriolic apparatus such as is often met with (Prowazek, 1909). Moore, Breinl and Hindle (1908) have described a similar structure, which

Minchin seems not to have noticed (1909). It is not always to be seen, sometimes only one chromatic granule is present.

Degenerated flagellates may sometimes be observed, in which the blepharoplast has lost its compact structure. In these cases the presence of an achromatic and chromatic substance becomes particularly clear (Diagram II). The blepharoplast is swollen and the two chromatic granules wide apart (fig. 1). Subsequently the chromatic part is broken up into many granules, still united by the swollen achromatic substance (figs. 2, 3). We shall have occasion to refer again to this degenerative hypertrophy of the blepharoplast.

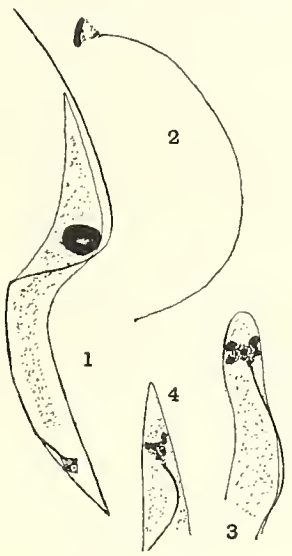

Diagram II.

Fig. 1. Swollen blepharoplast. Protoplasm showing "periplast line."
Fig. 2. Isolated flagellum with corresponding blepharoplast.
Figs. 3—4. Hypertrophied blepharoplasts.

(*c*) *The flagellum* seems often to be free from the blepharoplast. At its base the basal granule is frequently though not always to be seen. Sometimes an achromatic (pink) filament is seen uniting the base of the flagellum to the blepharoplast. This filament was mentioned by Rosenbusch (1909) and by Breinl, Moore and Hindle. In degenerated cells it may be seen that the flagellum and the blepharoplast are really connected: the blepharoplast has lost its definite structure, the protoplasm does not stain well; the achromatic part becomes particularly clear and is always united to the base of the flagellum (Diagram II). In normal cells where the protoplasm is well stained, this filament is

often invisible. In isolated flagella, which are sometimes to be found, the blepharoplast is nearly always present, the filament uniting flagellum and blepharoplast is very distinct in such cases. Minchin thinks that shrinkage, due to defective fixation, may separate the flagellum from the blepharoplast. If this connection were so delicate, one would never be able to find free flagella with their blepharoplasts, so I think this separation is due to some other cause.

(*d*) *The nucleus.* The nuclear structure has already been described in my previous paper. Giemsa (1910) holds that the old method of fixation and staining does not show the minute nuclear structure. Diagram III, figs. 1, 20, 21 show the same structure as Giemsa described. It is very common in preparations made according to the old method.

Diagram III.

Changes in the structure of the non-dividing nucleus.

The structure of the nucleus is not so simple as that described by Moore, Breinl and Hindle. The simplest form shows a central karyosomatic granule, united with the periphery of the nucleus by some achromatic filaments (figs. 1, 21). This central chromatic mass can completely disappear, so that only a central achromatic structure remains (fig. 20). The karyosome may also be divided (figs. 2, 4, 6—8) and the two parts may divide again (figs. 15, 16) or only one of them may divide (figs. 10, 14). If no division takes place curious deformation of these granules is observable, due probably to the fact that the chromatin is spread over the achromatic reticulum, so that X- or H-shaped structures are produced (figs. 9, 11—13); at the end the chromatin is dispersed more or less completely over the achromatic filaments of the nucleus, so that these filaments become very distinct and the chromatin seems to be present in the form of rodlets or of a zigzag line (figs. 22, 23). A third

change of structure is the production of a central clear space in the nucleus (fig. 3), the karyosome breaking up and forming a circle of chromatic granules round it. The granules may be equal or unequal (figs. 17—19).

Prowazek (1905) has interpreted some of these stages (such as figs. 6—8, 10, 13—16, 18) as "autosynthesis" of the nucleus, by which the karyosome divides in four parts, two of them degenerating and two combining to form a new karyosome (cf. Prowazek's figs. 5—8 with figs. 7, 14, 15, 18 of Diagram III). I think however that these forms selected by him out of a great variety of forms, have no more significance than any other of the nuclei of Diagram III so that I do not see that it is proved in the least that this autosynthesis really occurs. Minchin, who holds the same opinion, thinks that Prowazek's views are based on an incorrect observation due to a defective technique, but I think his observations (not the interpretation) are quite right.

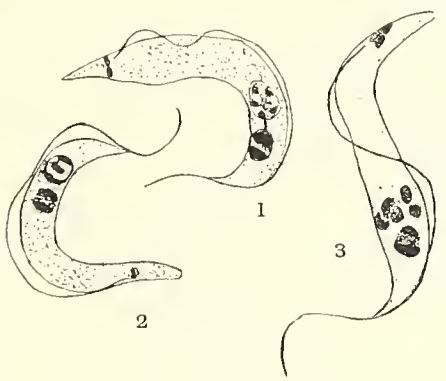

Diagram IV.

Figs. 1 and 2. Flagellates of the chronic stage of infection with two nuclei, one of them pycnotic.

Fig. 3. The same, but both nuclei pycnotic, one of them fragmented.

The peripheral granules of nuclei such as are represented in Diagram III, figs. 1, 20, 21, are generally regarded as chromosomes and their number is said to be constant. This is not the case in the nuclei shown in Diagram IV. I do not think them to be real chromosomes, because they do not play any part in the nuclear division.

Very large karyosomes nearly occupying the whole nucleus were never observed. I have already stated elsewhere the probability that they are due to shrinkage, the shrunken nucleus being taken for the karyosome (1910).

In the period of chronic infection, two nuclei may be observed in one cell (Diagram IV) sometimes united by a thin chromatic filament (fig. 1). One of the two nuclei is normal in structure, the other is always pycnotic and looks degenerated. This nuclear division is never followed by that of the cell, but it might be a reduction previous to the formation of gametes. Apart from these forms, we find others, where also a nuclear division takes place but where now two of them are obviously degenerated and fragmented (fig. 3), consequently I believe that these nuclear divisions are abnormal and to be regarded as pathological fragmentations.

(*e*) *Chromidia.* The study of the formation of chromidia is easier under abnormal than under normal conditions, consequently the subject will be more fully dealt with when treating of the abnormal morphology of *T. lewisi.* Flagellates containing chromatic granules may be frequently observed in rats' blood (Diagram V). Fig. 1 seems to indicate that

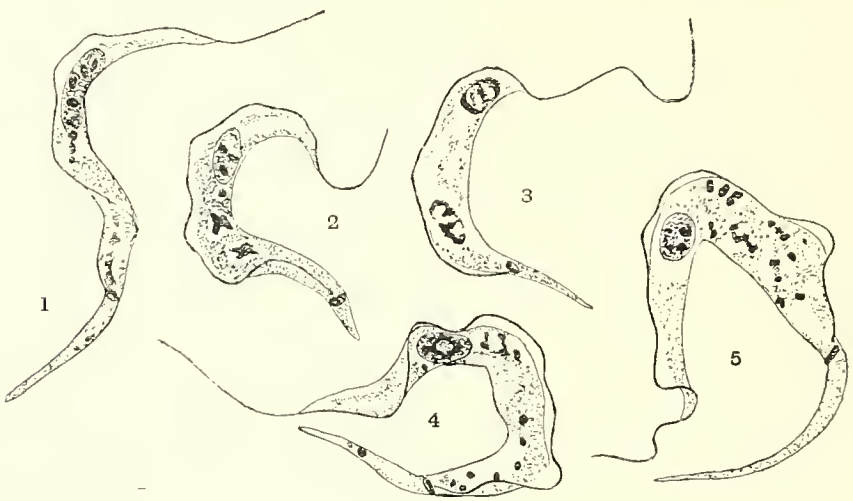

Diagram V.

Figs. 1—5. Different stages of the formation of chromidia.

the chromidia may be formed under the influence of the nucleus and the blepharoplast. In fig. 4 the two forms of chromidia are yet to be separated, although they have become more diffuse; in fig. 5 they have intermixed. Figs. 2 and 3 show an agglomeration of the chromatic substance, leading to the formation of one or few compact chromatic masses, sometimes resembling a nucleus (fig. 3). These figures are interesting because similar stages have been described as an autogamy by Moore, Breinl and Hindle, the blepharoplast emitting chromatin

which wanders towards the nucleus, the latter being previously reduced. Fig. 1 might represent the expulsion of the chromatin from the blepharoplast, figs. 2 and 3 the wandering towards the nucleus and fig. 4 the arrival of the chromatin near the nucleus. I believe, however, that fig. 5 seems to indicate that sometimes the chromatin may not reach its destination and may be dispersed on the way. The observations of the authors I have mentioned indicate only that the chromidia may be produced near the nucleus and the blepharoplast; but that this phenomenon has anything to do with an autogamy is not proved. Hindle (1909) seems to hold the same opinion regarding *T. dimorphon*.

Prowazek's "nuclear reduction" occurring in the blood of the rat must be regarded as a stage in the formation of chromidia.

As to the origin of these chromatic granules, I have no actual proof that they are really formed by the nucleus or the blepharoplast, although this seems to be probable judging from the phenomena observed in degenerating cells (see section III: abnormal morphology). Their chemical constitution could not be studied because they were not frequent enough to be found in normal cells.

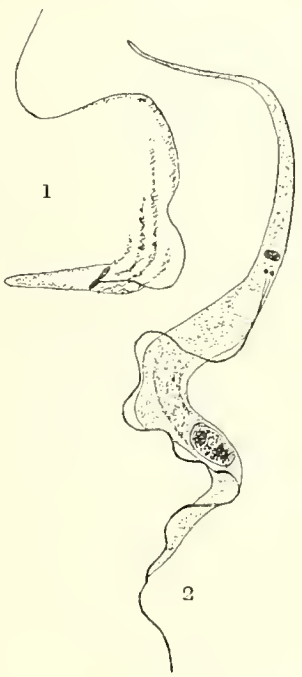

Diagram VI.

Fig. 1. Isolated periplast showing longitudinal striae.
Fig. 2. Trypanosome with long hindend and two striae.

(*f*) *The periplast* covers the protoplasm as a thin layer, which sometimes (especially in young cells) may be demonstrated as a delicate red line surrounding the cell. The periplast is more resistant than the rest of the trypanosome and is sometimes (with the flagellum and the blepharoplast) the only thing that remains of the flagellate (Diagram VI, fig. 1).

In such isolated periplasts longitudinal striae are sometimes to be seen, but whether they are myonemes or folds of the periplast I am not able to say, although the latter supposition seems the more probable. In normal cells I sometimes observed two longitudinal striae such as have been described by Prowazek (fig. 2) but this is all I saw of the complicated system of "myonemes" and "anchoring fibrillae" described by this author. Minchin doubts the existence of all these structures and I am inclined to share this doubt.

2. *Stages of division.*

(*a*) *Division of the cell.* (Diagram VII.) The normal mode of division, by formation of rosettes, has been described so carefully that it is not necessary to repeat this here (fig. 1). The products of this

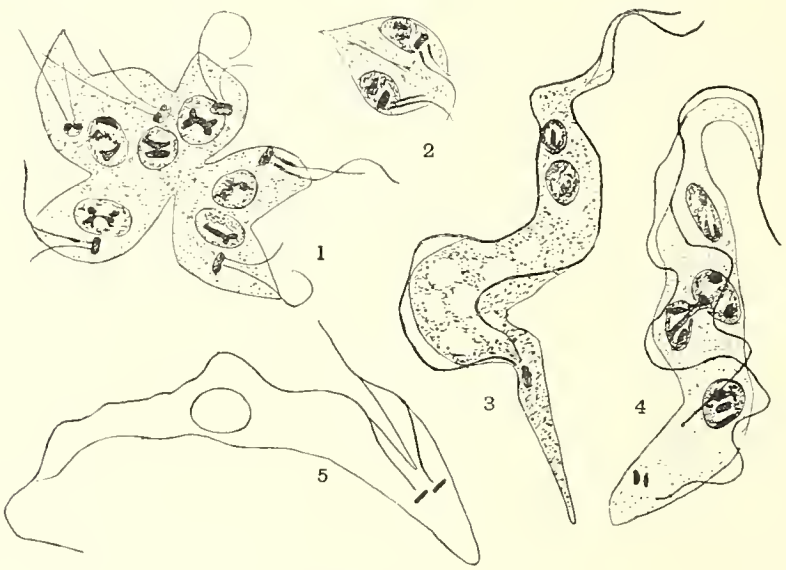

Diagram VII.

Fig. 1. Rosette.
Fig. 2. Dividing "leptomonas."
Fig. 3. Equal binary division.
Fig. 4. Equal multiple division.
Fig. 5. Unequal binary division.

division ("leptomonas-forms") may divide again before recovering the normal shape of a trypanosome (fig. 2). These forms have been called "latent bodies" by Moore, Breinl and Hindle, who seem to think that they are resistant bodies. Why they are called "latent" I cannot imagine, for they divide and move about, and are as active as ever they can be[1].

There may also occur (though not very often) an equal multiple division without the production of a rosette (fig. 4). Many nuclei and flagella are formed and the probable result is the production of flagellates of the normal shape and size, although I cannot say this with certainty because I only met stages as drawn in fig. 4. So far as I know this mode of division of *T. lewisi* has not yet been described. This multiple equal division leads to the binary equal division (fig. 3) already described by Swingle (1907). I never saw any stage but that shown of fig. 3, so I do not know whether division actually takes place at this stage or not. Perhaps fig. 3 represents only a stage previous to the one shown in fig. 4, so that both of them belong to the same mode of division. Fig. 5 shows unequal binary division, very seldom to be observed; I saw it only once.

(*b*) *Nuclear division.* (Diagram VIII.) Two types may be distinguished: (*a*) a division in which a karyosome seems to take a leading part; (*b*) a division where no central chromatic mass is to be seen.

Type (*a*) (figs. 1—6). The nucleus becomes elongated and the karyosome takes the shape of a dumb-bell (fig. 1); often, though not always, the two poles of the dumb-bell are united to the periphery of the dividing nucleus by radiate fibrillae. The karyosome divides (fig. 2), the nucleus becoming more and more elongated. The two karyosomes may divide again before the nuclear division is complete (fig. 3). A clear space now appears in the equatorial region of the nucleus (figs. 4, 5) which becomes more distinct and indicates the line of demarcation of the two nuclei; they become rounded at the edges and the two daughter-nuclei are constituted (fig. 6). Sometimes the division of the karyosome is delayed, so that the nucleus is practically divided at a time that the two daughter-karyosomes are yet united by a filament (fig. 4). I did not often observe these stages, but sometimes they may be frequent and then the two nuclei may be separated by a long distance, the karyosomes still holding together by a very elongated filament as is shown in the figures of Moore, Breinl and Hindle and as I myself observed in Prof. Minchin's preparations. It is worth men-

[1] Fig. 4 (Diagram X) shows a curious spur-like appendix to such a "leptomonas form." Its significance is unknown to me.

tioning that in stages like figs. 3—5 it is not uncommon to see the karyosome very distinctly in one half of the nucleus, whereas it is invisible in the other half.

Type (*b*) (figs. 7, 8). Here the nucleus becomes equally elongated (fig. 7) and in the centre a structure may be seen resembling the karyosome of fig. 1, only it is achromatic. Whether this structure must be considered as a karyosome which has lost its chromatin, or simply as

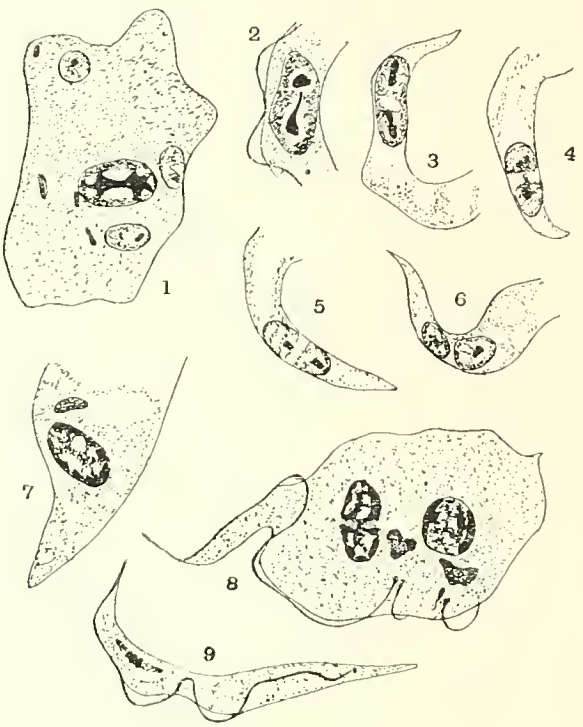

Diagram VIII.

Figs. 1—6. Nuclear division with karyosome.
Figs. 7—8. Nuclear division without karyosome.
Fig. 9. Pseudomitosis.

a part of the achromatic reticulum I do not know. In fig. 8 (nucleus on the left hand) the last stage of division is represented. The two daughter nuclei are already separated but still linked together by a filament uniting the two central achromatic structures. All the chromatin is situated at the periphery.

If this central achromatic structure is only a part of the achromatic reticulum supporting the chromatin, I seriously doubt whether a karyosome does really exist in the nucleus of *T. lewisi*. The figures of Diagrams III and VIII seem to indicate that the chromatin may wander

from the centre to the periphery (and *vice versa*). If it is assembled in the centre, a "karyosome" seems to be present (not only stained but also in the "standard preparations"), but if the chromatin goes to the periphery the "karyosome" disappears. Hartmann would perhaps interpret this phenomenon as an evidence of the cyclic properties of the karyosome, but I doubt whether this view is quite correct.

In preparations fixed with Flemming's fluid and stained with iron-hematoxylin, nuclei may be seen of the shape drawn in fig. 9. The nucleus has undergone considerable shrinkage, two chromatic granules fill up the equatorial region and two others are situated at the poles of the spindle-shaped nucleus. Similar structures were sometimes interpreted as mitotic division. These nuclei are only to be found after wet fixation (especially Flemming's), so I think these figures are artefacts produced by the considerable shrinkage sometimes following upon this mode of fixation.

It may be noted that in studying the structure of the nucleus and the blepharoplast with the help of Giemsa's stain it is necessary not to stain too long. In many cases the staining is just suited for the nucleus, at a moment when the flagellum is not yet stained.

(*c*) *Division of the blepharoplast.* (Diagram IX.) The division begins with the augmentation of volume of the achromatic substance (fig. 1 *a*). Then the whole blepharoplast is elongated (fig. 1 *b*), the

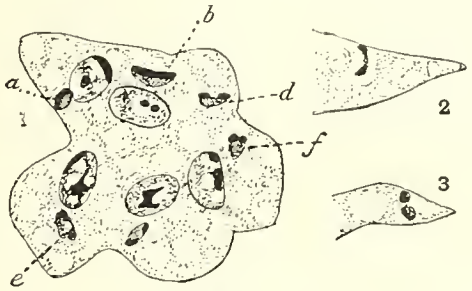

Diagram IX.

Figs. 1—3. Stages of the division of the blepharoplast.

chromatic substance becomes dumb-bell shaped (fig. 1 *e*) and presently is separated into two parts (fig. 1 *d*). The achromatic substance now becomes very much elongated (fig. 2) forming a regular "centrodesmosis." At last it is broken and the two small daughter-blepharoplasts are formed each with their chromatic and achromatic components. Sometimes the chromatic portion is divided in three parts (Diagram VIII, fig. 8) but

I do not know whether or not this leads to a triple division of the blepharoplast. In blepharoplasts where the chromatic portion is already divided (such forms occurring already in the chronic stage of infection) the two parts are only separated, but of course these forms cannot be distinguished from those resulting from a division of a blepharoplast with a single chromatic granule.

Prowazek considers the chromatic part of the blepharoplast as the karyosome. Fig. 1 seems to indicate that this part of the blepharoplast governs the division of the whole organellum.

I never saw any sign of mitotic division of the blepharoplast as was described by Rosenbusch (1909), but my description corresponds perfectly with that of Moore, Breinl and Hindle.

(*d*) *Formation of the new flagellum.* (Diagram X.) Moore, Breinl and Hindle affirm that the new flagellum is formed out of a granule arising from the blepharoplast. Laveran and Mesnil (1901) long ago stated that the new flagellum was formed by the separation of a part of the base of the old flagellum, this part growing out. This view, contradicted afterwards, is the correct one in my opinion.

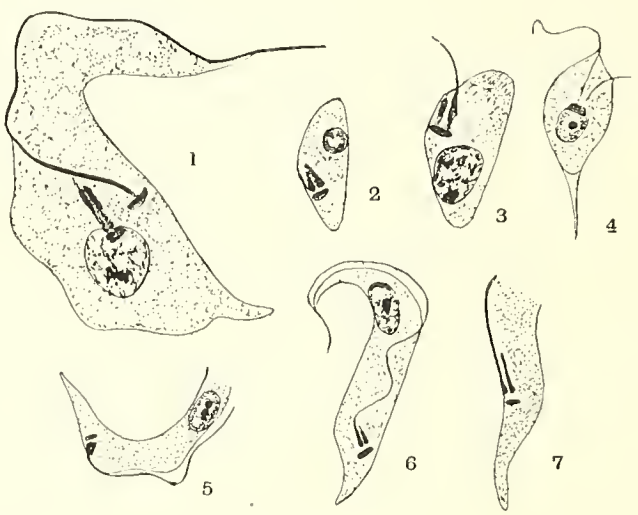

Diagram X.

Figs. 1—7. Production of a new flagellum.

This process is best studied in the "leptomonas-forms" and small trypanosomes arising from the division of larger forms. In these cells the flagellum continues to divide when still very small. In fig. 1 a new flagellum has been formed and is dividing again. There are two basal granules present, out of the left-hand granule arises a tiny flagellum,

the right-hand granule is still aflagellar. In this case the filament uniting the basal granule to the blepharoplast is very distinct, it shows only the beginning of a longitudinal division, its fission follows that of the basal granule. This figure shows that the flagellum is divided before the blepharoplast. Fig. 2, a "leptomonas," shows the same as fig. 1, only here no connection is to be seen between basal granule and blepharoplast. Here the right-hand granule produces a flagellum which as yet is very short. It shows that there is no sharp differentiation between the flagellum and the basal granule, but that the latter is only the thickened basal part of the first. Fig. 3 is similar to fig. 2 only here the right-hand granule is more developed. Flagella in different stages of growth may be seen also in figs. 1 and 2 (Diagram VII). Fig. 4 shows how the undulating membrane is produced, the flagellum not piercing the blepharoplast, but pushing it forward, when growing out (fig. 4).

Figs. 5—7 show the same phenomena in small trypanosomes, fig. 5 shows the division of the basal granule, figs. 6 and 7 the growing out of the new flagellum.

The thickened base of the flagellum (basal granule) is consequently the active part of this organellum; it divides and by growing out produces a new flagellum. The blepharoplast has nothing directly to do with it, except that the basal granule is united with the latter and so in a certain sense forms a part of it. The basal granule was never seen to be formed by the blepharoplast, nor did it produce the flagellum by a division (as is the case in *T. noctuae*).

It seems that the basal granule, which is very distinct during the division of the flagellum, disappears after division is over. This seems to indicate that it is not an independent organellum as are the blepharoplast or the nucleus but only a part of the flagellum itself, endowed with a special function.

(*e*) *Different forms of the trypanosomes during the period of division.* (Diagram XI.) During this period there is a marked polymorphism among the full-grown cells, affecting the length and breadth, the undulating membrane, the free flagellum and the way in which the protoplasm is stained by Giemsa.

Figs. 1 and 7 are two extreme types: fig. 1 short and broad, with a short free flagellum, the protoplasm staining dark blue; fig. 7 slender, spirochaetiform, with a long free flagellum and protoplasm staining violet. The first trypanosome is of the "female" type, the second of the "male" type. Between these two are all sorts of intermediate forms (figs. 2—6), so I do not think that in this case the dimorphism has

anything to do with sexual differentiation, but may be explained by the fact that such forms as figs. 1 to 3 are about to divide[1].

In this period the curious long-tailed forms, mentioned already, are also to be found (Diagram VI, fig. 1).

Diagram XI.

Polymorphism of full-grown cells during the period of division.

(*f*) *Autogamy.* In a recent article Schilling (1910) describes some changes in the relative position of the nucleus and the blepharoplast in dividing forms of *T. lewisi.* He holds that these changes indicate an autogamy. The blepharoplast comes near to the nucleus and seems to come in contact with the karyosome for a short time.

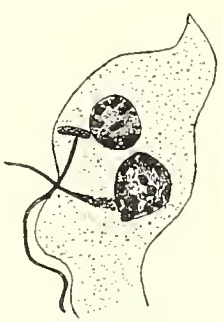

Diagram XII.

"Autogamy."

[1] This explanation of the dimorphism does not apply to *T. gambiense* and *dimorphon* Here we have perhaps a real sexual differentiation.

I have seen similar changes, only the nuclei of my preparations do not possess such large karyosomes as Schilling has drawn. Minchin (1910) and I (1910) have shown elsewhere that these karyosomes are artefacts; Diagram X, fig. 4 shows the beginning of the autogamy, Diagram VII, fig. 2, Diagram X, fig. 1 and Diagram XII the subsequent stages. They can all of them be explained more naturally by superposition. So it is in Schilling's figures, except fig. 2, but there it is by no means proved that the intranuclear granule near the karyosome has anything to do with the blepharoplast. I think that the existence of autogamy is not demonstrated by Schilling's observation.

3. *Abnormal morphology.*

It is known that by preserving blood containing *T. lewisi* in the refrigerator, the trypanosomes may survive for a considerable time (up to 20 days). During this time they become agglomerated, the protoplasm becomes granulated and at last they degenerate completely. No attempt has been made to study systematically the different morbid changes occurring in trypanosomes under these circumstances, although this study is important so as to be able to interpret correctly the different forms found in the gut of invertebrate hosts (lice, fleas, bugs, ticks). In the paper by Strickland and myself (1910) we showed how many abnormal forms are found in the flea's gut, etc. shortly after the infecting meal, before the real development begins. These forms have been interpreted by some authors (*e.g.* Prowazek) as sexual forms. I will try to show that similar forms are to be found in the blood preserved in the refrigerator, so that probably the corresponding forms of the invertebrate hosts must be regarded as degenerative.

The blood was kept for 7—11 days in the refrigerator in capillary tubes (mixed with a 0·5 % solution of citrate of sodium in 0·5 % saline). After 11 days the changes to be described here were well marked, after that time all the trypanosomes died and were dissolved except the flagella, so my description refers mainly to forms preserved for 11 days in the refrigerator. The different changes mentioned here occur all ante-mortem.

(*a*) *Changes of the blepharoplast.* (Diagram XIII.) The blepharoplast becomes hypertrophied, sometimes the achromatic substance seems to free itself from the chromatic part (fig. 3), the latter may be divided in several portions (fig. 2). Also the achromatic part can become much elongated and curved, the chromatin distributed over it

as minute granules (fig. 4). Sometimes the whole blepharoplast is divided in two unequal parts (fig. 7), the flagellum being sometimes connected with the smaller one (Diagram XIV, fig. 6). Equal division may also take place and the two parts may divide again (fig. 5). All these equal or unequal divisions are continued (figs. 6, 8—10) so that at the end it is difficult to say where the true blepharoplast is situated,

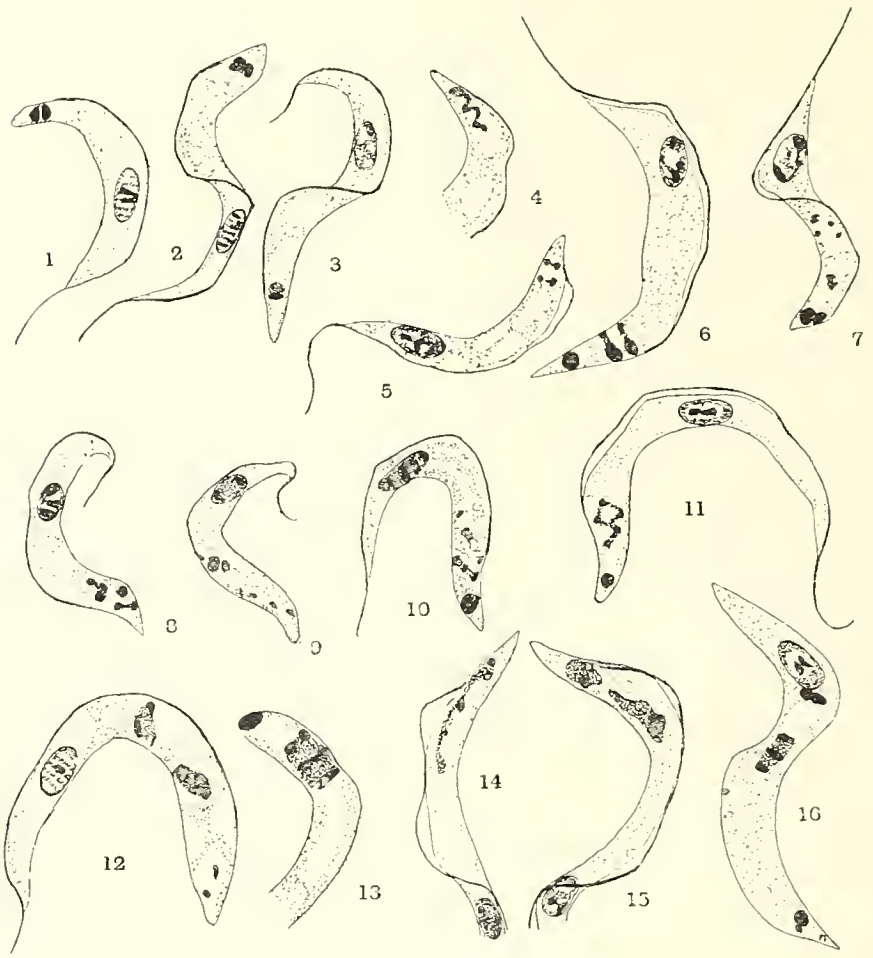

Diagram XIII.

Figs. 1—4. Hypertrophy of the blepharoplast.
Figs. 5—8. Division of the blepharoplast.
Fig. 9. The blepharoplast wanders towards the nucleus.
Fig. 10. Pseudomitosis of the blepharoplast.
Fig. 11. Reticular chromidia near the blepharoplast.
Figs. 12—13. Formation of large chromatic masses.
Figs. 14—16. The blepharoplast emits chromatic substance which wanders towards the nucleus.

especially so because the base of the flagellum becomes often very indistinct. Many dividing granules are to be observed which often look very regular and suggest a "reduction" of the blepharoplast (figs. 5, 6, 8, 9) or even mitotic spindles are simulated (fig. 10). Sometimes the blepharoplast, and with it the flagellum, wanders in the direction of the nucleus (fig. 9), but a real crithidia facies is never produced. In other cases the products of hypertrophy and division of the blepharoplast are less regular and take the shape of achromatic reticula with chromatic granules distributed over it (fig. 11). All these changes may be produced on a much larger scale, large masses of chromatic and achromatic substance being formed (figs. 12, 13) or long chromatic strings being loosened from the degenerating blepharoplast (figs. 14, 15). Some of these chromidia, as they may correctly be named, wander towards the nucleus, so repeating on a large scale Moore, Breinl and Hindle's "autogamy."

Many of the changes of the blepharoplast described here bear a marked resemblance to the reduction of the blepharoplast during the parthenogenesis seen by Prowazek in cultures, and previous to copulation in the gut of *Haematopinus*. I think all these phenomena do not belong to the normal life cycle of *T. lewisi*. As in the blepharoplasts of fig. 3 (Diagram II) the distinction of chromatic and achromatic substance is well marked in these degenerating blepharoplasts.

(*b*) *Changes of the nucleus.* (Diagram XIV.) The nucleus becomes first enlarged and the karyosome becomes often elongated as during a division (fig. 1); then it becomes larger and seems to absorb all the chromatin, the rest of the nucleus becomes pale and indistinct (fig. 2). The karyosome becomes longer and takes the shape of a twisted filament, the rest of the nucleus becoming indistinct (fig. 7) and disappears at last (fig. 8), so that instead of a nucleus there appears a filament, wound in the form of a spiral or zigzag line (figs. 9, 14) closely resembling Prowazek's "male" form.

In other cases the chromatin may be expelled from the nucleus (fig. 3) in the form of one or more granules or irregularly shaped masses (figs. 4—6). These forms correspond to the stages of nuclear reduction in the rat's blood and in the louse's gut as described by Prowazek. If these were the only stages to be found, this supposition might be possible, but the formation of chromidia continues (fig. 10), the nucleus becoming pycnotic at the same time. At last only some dumb-bell shaped rodlets are to be found and when the blepharoplast is broken up at the same time (which is not always the case), the cell is filled with

scattered remains of the nucleus and the blepharoplast (figs. 11, 12, 14)[1].

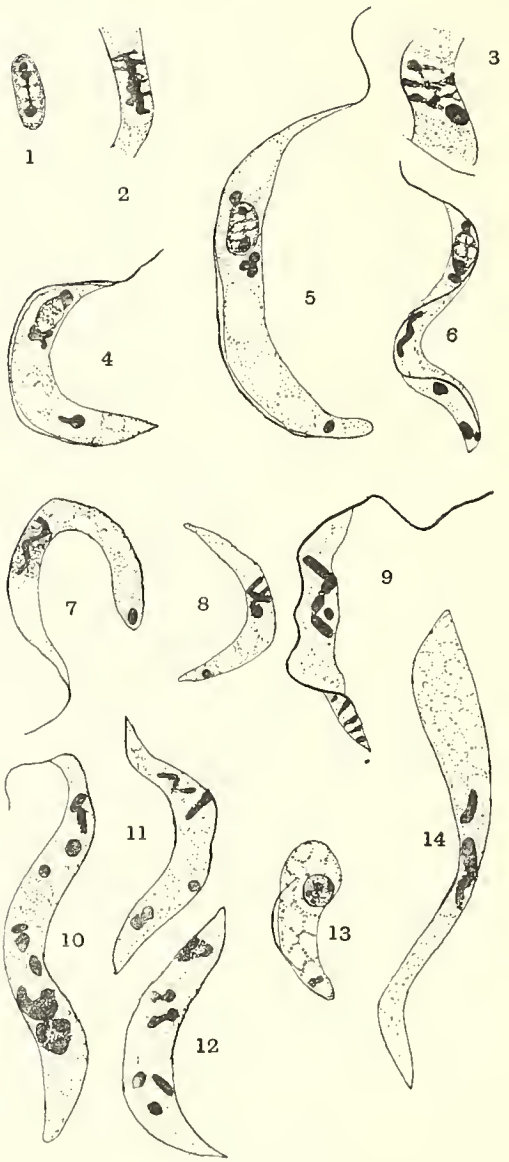

Diagram XIV.

Figs. 1—3. Karyolysis.
Figs. 4—6. Formation of chromidia.
Figs. 7—9, 14. Formation of a chromatic filament.
Figs. 10—12. Complete destruction of nucleus and blepharoplast.
Fig. 13. Round cell with normal nucleus and blepharoplast and short flagellum.

[1] These chromidia are not composed of "volutin" as might be expected, after what is known in other Trypanosomes (*e.g. T. brucei* and *gambiense*).

It would take too long to compare in this paper all the stages of degeneration with Prowazek's figures of nuclear reduction, gametogenesis, etc., but if the figures are compared, it will be seen that they are remarkably alike and that their resemblance cannot be explained as merely due to coincidence.

(*c*) *Changes of the shape of the cell.* (Diagram XIV, fig. 13.) Little is to be said on this point. Cells as shown in fig. 13 were not commonly seen. Perhaps these forms are to be compared with Moore and Breinl's "latent bodies," but this cannot be affirmed with certainty.

It might be argued that these degeneration forms and the stages of development found in the gut of *Haematopinus* are not to be compared, although bearing some superficial resemblance to each other. This, however, is not the case. As Strickland and I have shown and as may be seen also in Diagram XV, both the same phenomena, viz. hypertrophy

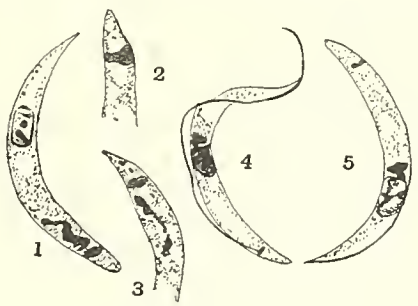

Diagram XV.

Degenerating *T. lewisi* in the gut of Hæmatopinus spinulosus.

and fragmentation of the blepharoplast (figs. 1—3) and formation of chromidia and fragmentation of the nucleus (figs. 4, 5), are also to be found in trypanosomes in the gut of the rat-louse, if no further development takes place.

4. *Summary.*

The development of *T. lewisi* in the blood of the rat is accompanied by a division of the nucleus which is more like an amitosis than a mitosis.

The division of the blepharoplast, always preceded by the division of a karyosome-like granule, might be called a promitosis (*sensu*: Nägler, 1909).

It is not proved that autogamy or parthenogenesis takes place in rats' blood or in cultures. The evidence brought forward by Prowazek

and by Moore, Breinl and Hindle proves only the existence of chromidia; neither are Schilling's recent proofs suggestive.

Under abnormal conditions hypertrophy takes place affecting the nucleus and the blepharoplast, with subsequent formation of chromidia and final fragmentation. Similar stages found in the rat-louse have been described as sexual cells.

Considering these results in connection with previous observations of Strickland and myself, we may conclude that as yet nothing whatever indicates the occurrence of any sexual process in the life-cycle of *T. lewisi*, both in the vertebrate and invertebrate hosts.

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THE INFLUENCE OF TEMPERATURE UPON THE BITING OF MOSQUITOES.

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(With 4 Text-Figures.)

The fact that no inquiry seems to have been made into a matter of some general interest, namely, the reasons which, apart from "hunger," induce mosquitoes to bite, may serve as an excuse for presenting the preliminary results of an investigation which has had, for the time being, to be discontinued. The following observations on mosquitoes were made at the Pusa Research Institute.

Among common Indian mosquitoes *Stegomyia scutellaris* is one of the most fearless biters. Five or six females of this species (unfed) were placed in a wire breeding-cage, and into the cage was introduced a glass slide on which were several drops of fresh blood forming a little pool about the size of a shilling. They seemed to take no notice of the blood. Other drops of blood were presented to single mosquitoes in tubes, with a like result.

A test-tube half-full of perspiration taken direct from the skin was placed on a low shelf in a room where *Culex fatigans* and *S. scutellaris* were present in some numbers: the time was late afternoon and the mosquitoes were biting, but an hour's watch gave no indication that they were particularly attracted by the contents of the test-tube. On the following day a small glass beaker was filled with perspiration wrung from a cotton shirt, and was placed on the same shelf, where it was left for three days. It was inspected at fairly frequent intervals, especially in the afternoon and evening: one or two *C. fatigans* were seen to suck moisture from the edge of the beaker, but this they will do with pure water, and apart from this nothing was noticed which would

encourage the idea that the smell of the sweat was attractive either to *C. fatigans* or to *S. scutellaris*.

The following are some of the attributes of a living warm-blooded animal which may be supposed to advertise its presence to an insect such as a mosquito.

(1) *Motion.* Probably unimportant. Mosquitoes bite a motionless sleeper.

(2) *Form and colour.* They are very obviously attracted by black and dark colours (it is remarkable that tsetse-flies are similarly attracted by black), but pay no regard to form as far as can be seen. The fact that black objects are good absorbers and good radiators of heat may be not without significance (see below).

(3) *Smell.* Blood and sweat without apparent influence.

(4) *Temperature* and air-currents due to the motion of air warmed by the body.

With regard to this last (No. 4), it is obvious that the lighter an insect is, or rather the greater its surface area in proportion to its weight, the more susceptible to air-currents is it likely to be. Since the volume (and therefore the weight) of solids varies as the cube, and the surface-area as the square of the linear dimensions, the ratio $\frac{\text{surface}}{\text{weight}}$ for two insects of the same shape will be, roughly speaking, inversely proportional to their respective lengths: that is to say, an insect 2 mm. long will expose five times as much surface in proportion to its weight as a

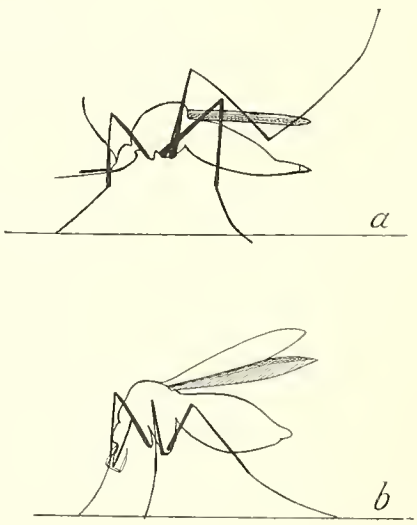

Fig. 1. Resting attitude of (*a*) *Mucidus scatophagoides*, (*b*) *Phlebotomus babu*, both from photographs (not drawn to scale).

similar insect 10 mm. long. A very gentle breath of air stimulates fleas to great activity. *Phlebotomus* seems peculiarly sensitive to air-movements, and to this sensitiveness its small size, curious resting-attitude, long legs, and long hairy coat probably all contribute (Fig. 1). Mosquitoes have similar characters in a somewhat modified form, and they are exhibited to some extent by many of the *Chironomidae* and by other non-bloodsucking Nemocera, but not by *Tabanidae,* most *Muscidae* (including *Glossina*) or *Hippoboscidae.*

On more than one occasion when mosquitoes have been troublesome at tea-time I have noticed that they seemed to be fond of hovering in the neighbourhood of the tea-pot, being attracted apparently by the heat: moreover, I have heard it said that during attacks of fever (when the temperature of the skin may be raised above the normal) mosquitoes are apt to be more than usually vicious. My own experience rather tends to confirm this notion, but of course such an idea may arise merely from an increased susceptibility to small annoyances.

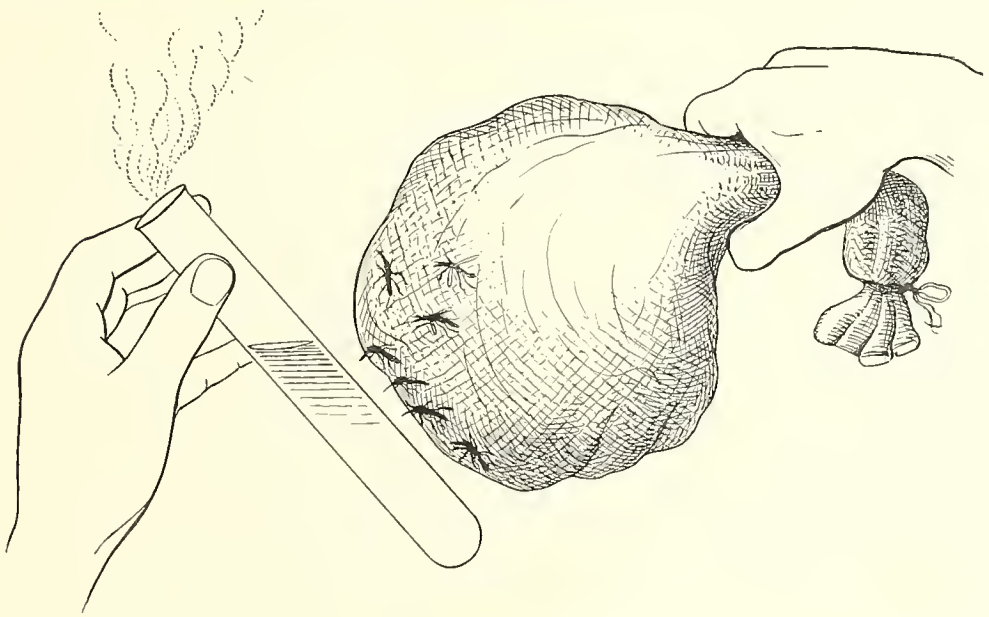

Fig. 2.

In order to try the effect of heat, several females of *S. scutellaris* were placed in a loose gauze bag, and a test-tube half full of hot water was presented to them in the manner shown in Fig. 2. The effect was most interesting. As soon as the hot air from the tube reached the insects they became restless and began to congregate on the part of the net nearest the tube; as this was brought nearer they became more

excited, stabbing with their proboscides through the meshes of the net and displaying the utmost eagerness in their fruitless efforts to puncture the glass. The females of *C. fatigans* responded in a similar way, but no reaction at all was obtained when the males were tried with the hot tube, either with *C. fatigans* or *S. scutellaris*, and when males and females were mixed in the same net the difference in the behaviour of the sexes was very noticeable.

Neither males nor females paid any attention to the tube when it was filled with cold water. To give the two tubes an equal chance, they were arranged in a narrow glass cell as in Fig. 3, but this made no difference, the insects always going to the hot tube, though with less eagerness than when the net was used: this may very probably have been due to absorption and conduction of heat by the metal gauze. Though there were some differences in the degree of eagerness shown, results essentially similar were obtained with the females of *Myzomyia rossii*, *Nyssorhynchus fuliginosus*, *Mucidus scatophagoides*, *Stegomyia gubernatoris*, and *Stegomyia sugens*.

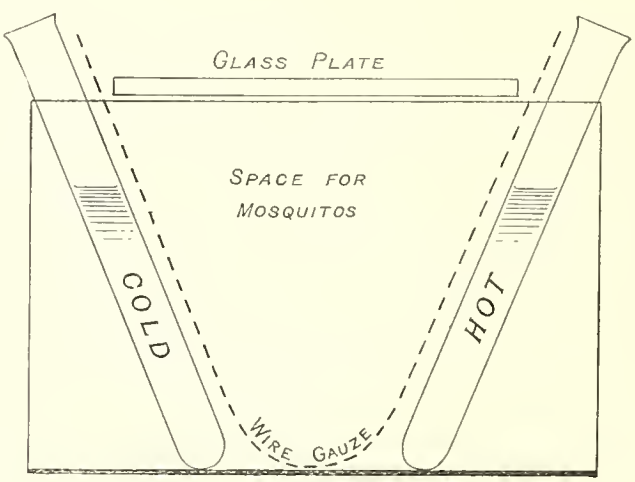

Fig. 3.

Only in one case did the presence of the tube produce little or no effect. This was with a single female *Stegomyia thomsoni*, the only one available for experiment, and it is interesting to note that my assistant Mr Sharma failed after several attempts to induce this individual to suck blood from his arm, and was of opinion that its proboscis was not strong enough for the purpose: its movements certainly looked as though such might be the case.

The effect of the hot tube was very much less when it was held as in Fig. 4 in such a way that most of the hot air from its surface passed clear of the insects, indicating that it is the current of heated air and not the radiant heat from the tube which exerts the more influence. The eagerness with which the mosquitoes attacked the tube slackened as the water cooled.

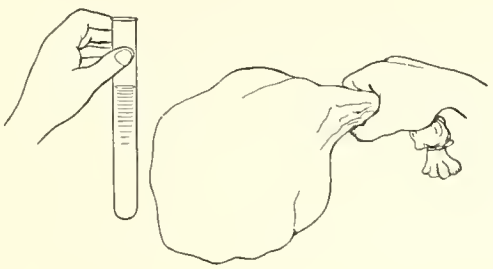

Fig. 4.

One or two easily observed facts may be considered in conjunction with the above results.

Mosquitoes often hover in a cloud over one's head, but this cloud is always columnar in shape, never flattened, and is well seen only when the air is still and the currents from the body are rising straight up from it. Such clouds are most often noticed towards the cool of the evening, when mosquitoes may also be seen hovering over almost any hot body, particularly if its form is such as to concentrate the rising air into a fairly narrow column.

Mosquitoes rarely attack Amphibians and Reptiles, while among warm-blooded animals birds (whose body-temperature is above 100° F.) are I believe quite as subject to attack as are Mammals, particularly if we take into account the relative degree of protection afforded by feathers. Horses are often much annoyed by mosquito bites.

With at least one notable exception (*S. scutellaris*), mosquitoes are nearly always most active at night or when the atmosphere begins to cool down towards sunset; I am speaking now of the Plains of India. In the hills where the air temperature is lower, I have fancied that their active period was much less definite and restricted, but this is merely a general impression based on small experience. The extreme ferocity of mosquitoes found in very cold climates is notorious, and has never really been explained.

The conclusions we may draw from the observations and experiments here outlined are (*a*) that the bite of a mosquito is a reaction to the stimulus provided by a hot surface, (*b*) that the mosquito is attracted to

the hot surface mainly by the warm air rising from it, and (*c*) that the strength of the reaction is, within certain limits, proportional to the "differential temperature" of the surface, *i.e.* the difference between its temperature and the general air-temperature at the time, and that this difference must be positive.

If this last conclusion is correct we should expect to find that mosquitoes bite only when the shade-temperature is below the body-temperature of the victim. My own imperfect recollections incline me to the belief that this may actually be the case. Careful observations carried on for a month or two in hot weather would probably give fairly conclusive evidence on the point, and would enable us to estimate the relative importance of the differential effect and the general increase in energy often associated with a rise of temperature.

While it seems certain that temperature is a dominant factor, it is more than possible that other influences may affect mosquito-attacks. Such are, for instance, light, the pressure, humidity, and electrical condition of the atmosphere, to say nothing of the physiological condition of the various organs and tissues of the insect itself. None of these have been taken into consideration. It would be rash to deny any importance to the sense of smell, in view of the vital influence it exerts on the lives of some other Diptera, and of the protective action of kerosene, citronella, and many essential oils when rubbed on the skin. An interesting point is the alleged comparative immunity from mosquito-bite enjoyed by certain individuals.

Study of the blood-sucking Diptera leads one more and more to the conclusion that in many respects they may be justly defined as mere "bundles of reflexes." The result of actual observation, as well as general phylogenetic considerations, make it extremely improbable that their reactions are of an identical nature throughout the different families, and their investigation from this point of view might well lead to some improvement in methods of control.

ON THE COLLECTION AND PRESERVATION OF INSECTS.

By F. M. HOWLETT, B.A., F.E.S.,
Christ's College, Cambridge. Second Imperial Entomologist, India.

(With 3 Text-Figures.)

The collection and study of tropical insects now being undertaken in connection with medical and other researches has resulted in the publication of two or three very useful leaflets of instructions as to how to catch and send home specimens of blood-sucking and parasitic insects. These leaflets are intended for collectors who will send home their specimens soon after they have caught them: when this is impossible, when specimens have to be carted from place to place and housed, it may be, in very unsuitable quarters, it becomes important to use only the best methods for preserving them in good condition, and the best methods can be arrived at only by combining the experience of collectors in different countries.

One of the greatest wants at present unsupplied is of a store-box which shall be really reliable in any climate. No wooden box seems able to stand with any certainty against the great variations of humidity so frequently experienced. At Pusa (Bengal) well-seasoned teak boxes are found to be reliable only when the wood is so thick as to render them unpleasantly heavy and cumbersome, a great disadvantage in a tropical climate. A metal box gets too hot; it cannot be left in the sun even for a few minutes, and at any drop in temperature moisture tends to condense on the inside. Cardboard "carton" boxes sometimes keep their shape for a surprisingly long time, but have no pretensions to permanence. Boxes of three-ply wood appear to be heavy and expensive: I have had no personal experience of them.

The ideal box would be (1) light, strong, and easily handled, (2) made of impermeable non-conducting and non-hygroscopic material, (3) unaffected by any degree of moisture or drought in conjunction with any temperature between 30° and 120° F., (4) ant-proof and reasonably air-tight, (5) not expensive. It should not be difficult to make such a box, if not of wood or impregnated wood, then perhaps of metal covered with some non-conducting substance, or of one of the many patent materials and "substitutes" now available. Single boxes are preferable to double ones.

The lining of boxes is usually of cork, cork carpet, German peat, or pith. The three latter tend to attract moisture more than does cork, and in time corrode the pins, unless these are made of silver or nickel. The lining should be of a fair thickness; for sending specimens by post an inch thick layer of pith is excellent; if the pith is procured locally it should be very carefully dried. Carbolic acid cannot be used with cork carpet or German peat, as it causes them to swell up in billows and crush the specimens against the lid of the box. Dr Graham of West Africa recommends white beech creosote as a preventive against mould and insects: Lefroy finds that an improvement on this is effected by mixing the creosote with an equal quantity of a saturated solution of naphthalene in chloroform; a little of the mixture is poured over the bottom of the box from time to time: it is very effective, and can be used with any lining.

The necessity of poisoning boxes is to a great extent if not entirely obviated by the use of paraffin-wax, which has been tried at Pusa with very good results. A box is lined with cork carpet painted white, and over this is run about a quarter of an inch of wax with 5 % of naphthalene melted in with it. The melting-point of the wax should be about 20° F. above the maximum shade-temperature. No "mites" seem able to live in these boxes. Both paraffin and the creosote mixture are superior to naphthalene alone, apart from the risk of damage to specimens occasioned by using lumps of the latter in the box.

It is possible to preserve diptera *very* carefully packed in layers of well-sifted sawdust, but this should never be done if it is possible to pin them. *Nothing but silver or nickel pins should ever be used,* and the insects should not be pinned directly in the box, but to a support which in turn is pinned firmly on to the lining of the box.

The round discs of paper so often used as supports for mosquitoes and other diptera have proved in our experience to be unsatisfactory, and

particularly so when used by people who have not had much practice in setting insects; their only advantage is that they are easily made. It is far preferable to use round or rectangular slabs of pith, papered or unpapered cork, or thin strips of polyporus: any of these gives a firm hold for both pins and will stand comparatively rough usage. It is difficult to get cork free from holes, and as an improvement on papered cork we have used cork carpet or "suberit" covered with an elastic quick-drying enamel called "Paripan" and cut to standard sizes. The supports should be pinned in the box with good stout pins, not thin elastic ones, and should not be put higher than about half-way up the pin; this gives protection to the specimen and makes it easier to handle. It is generally very much easier and quicker to pin specimens through the side than through the back, and great care should be taken that no part of the insect overlaps the support. If possible a small label bearing the date and locality should be gummed or pinned on the support, so that it can be seen at once: other labels at the bottom of the pin.

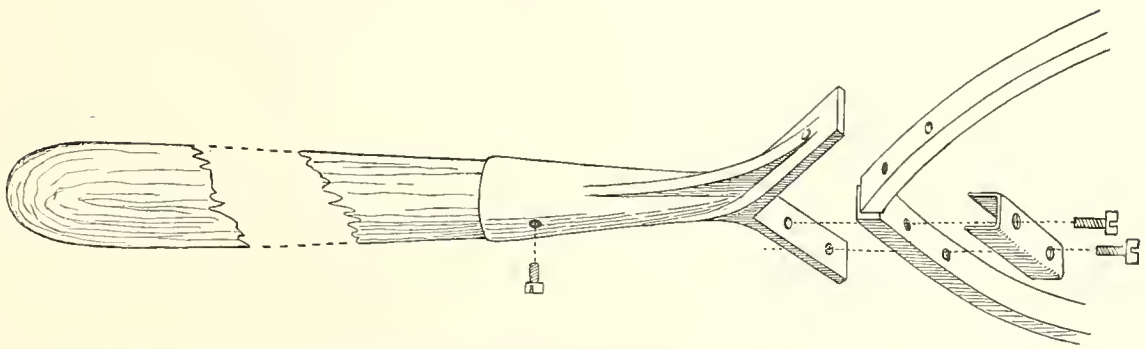

Fig. 1. The Y-piece is a solid aluminium casting, the hoop of wood, the screws of brass with deep and broad cuttings in the screw heads.

When pinned in the box the supports should be all at one uniform level.

The best pattern of net for hard work is that used at Cambridge (Fig. 1). The "Swiss bolting-silk" of which the bag is made is expensive, but quite worth its cost in the long run, as it is extremely durable and improves with wear: it should be well soaked and crumpled before use. Mosquito-netting and a wire ring does very well for mosquitos, but for *Simulium* and particularly for *Ceratopogon* a very small mesh is necessary. Messrs Winkler and Wagner sell a handy little pocket-net (kleines Fangnetz), but the bag is of such delicate material that it soon wears out. An old tennis-racket makes quite a good net-frame.

For killing, cyanide is perhaps most generally useful: benzene is apt to make the tubes sticky, and chloroform tends to stiffen insects killed with it, but either is useful in damp seasons when the deliquescence of cyanide may become a nuisance. It is much better to use several fairly small tubes than one large and heavy killing-bottle.

The diagram (Fig. 2) gives a suggestion for a tube which could be used with any killing-agent and be easily re-charged. If cyanide and

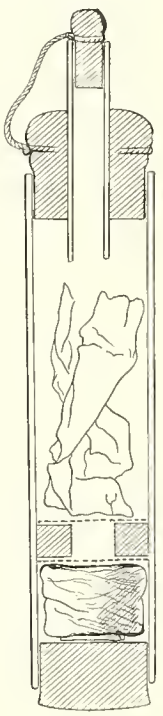

Fig. 2.

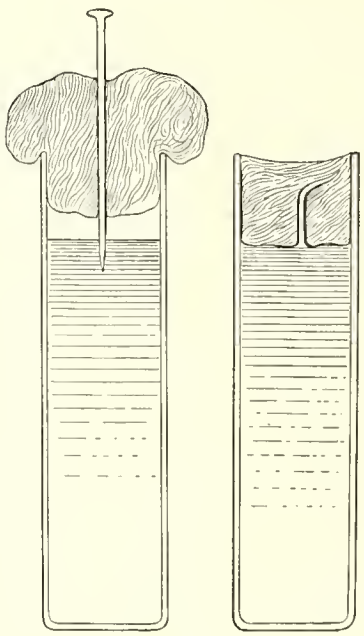

Fig. 3.

Fig. 2. The cartridge is seen below separated from the main tube by two layers of gauze seccotined on to a ring of cork.

plaster of Paris is to be employed, a piece of tube of similar bore is used as a mould in which a cyanide "cartridge" can be cast, the tube being lined with paper, and the cartridge when set pushed out of the tube and inserted in the killing-tube. Small lumps of cyanide wrapped in soft paper may be used instead, or cotton-wool wetted with benzene or chloroform. Rubber corks are attacked by the two latter substances, but not by cyanide. When insects have been killed in rainy seasons it is better to put them with some pieces of soft paper into pill-boxes rather than into glass tubes, as they run less risk of getting wet and spoilt. A number of living insects in a tube with paper will often keep

their condition (as regards moisture) better than the same number of dead ones, and if a certain degree of over-crowding is unavoidable, it is sometimes better merely to stupefy the insects and let them revive in a box or tube, and to kill at the journey's end. If of fair size, a little pad of soft paper above each (dead) insect is useful in absorbing moisture.

For closing tubes or bottles containing formalin-specimens, Mr Gordon Merriman has shown me a method which is both ingenious and efficient (Fig. 3). A plug of plasticine (cheap and easily obtained) is pushed right down on to the surface of the liquid, until the latter spurts up through a pin-hole made in the plug, all the air in the tube having previously escaped through the same hole. The hole is then sealed by a wipe of the thumb, and the liquid remains completely filling a permanently closed tube, preventing all damage from shaking. For closing bottles which contain specimens in any fluid preservative, and which will not require to be opened for a long time, "Viscose" bottle-caps (obtainable from chemists) seem admirably adapted, but they must be kept in a special fluid solution till used.

AUTHOR'S REVIEW.

THE DEMONSTRATION OF TRYPANOSOMES IN CATTLE

in Kreis Oberwesterwald by means of cultures in Blood-bouillon. *Berliner Tierärztliche Wochenschrift*, No. 27, pp. 539—540 (1910).

By KNUTH, RAUCHBAAR AND MORGENSTERN.

By adopting the method of Martini, in Manila, and Crawley, in North America, the authors succeeded in demonstrating the presence of Trypanosomes in the blood of 7 out of 25 apparently healthy cattle coming from various places in Kreis Oberwesterwald. The examination was made by collecting a small quantity of blood aseptically from the jugular vein, defibrinating it, and adding it to ordinary sterile beef bouillon. The inoculated tubes were kept at room temperature, being protected as much as possible against light. On being examined after a few days, numerous developmental and agglomeration forms of Trypanosomes were found in the tubes. The authors consider the assumption justified that animals suffering from a latent Trypanosome infection, when attacked by other diseases, such as foot-and-mouth disease, are likely to suffer more severely than animals free from Trypanosomes. Perhaps the apparently higher virulence of certain outbreaks of diseases in animals in certain localities may be referred to this cause. This method of examination for Trypanosomes may be of use in the investigation of certain other animal diseases whose cause remains as yet obscure.

Transmission experiments with the cultural forms of the Trypanosomes are at present being carried out in the Hygienic Institute of the Veterinary School in Berlin. It will be interesting to try and discover the mode of infection in nature, and the possible carrier of the disease in Germany.

The results of the investigations will shortly be published *in extenso*.

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for Bergman, Torberno
read Bergman, Torbern

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